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EDITED BY

SIMON FLEXNER, M.D.

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THE DETAILS OF THE PHOTOGRAPHICALLY RECORDED VENOUS PULSE IN AURICULAR FIBRILLATION.

By WALTER L. NILES, M.D., and CARL J. WIGGERS, M.D.

(*From the Second Medical Division of Bellevue Hospital and the Physiological Laboratory of Cornell University Medical College, New York City.*)

Plates 1 to 4.

(Received for publication, September 15, 1916.)

INTRODUCTION.

The condition of auricular fibrillation is easily diagnosed by the aid of arterial and jugular tracings or by electrocardiograms, and in most cases it can be recognized by careful physical examination alone. It is not possible, however, to interpret all the waves found in the venous records. Generally, these tracings may be placed in one of two classes; (*a*) those in which prominent systolic waves, differing in contour from the small, impact waves of the normal phlebogram, predominate or occur alone, and (*b*) those in which large or small diastolic waves recur occasionally, in groups, or in a continued series averaging 250 to 500 per minute.

The prominent systolic waves, designated as ventricular waves, have generally been attributed to an associated tricuspid regurgitation. Lewis[1] states: "'The ventricular form of venous pulse' is a term which expresses the only fixed quality manifested by graphic records taken from the jugular veins in these cases. . . . The older conception, that the prominence of the venous pulsation is an index of the degree of tricuspid reflux, is not without a definite foundation." The inference follows that tricuspid regurgitation is a frequent accompaniment of this form of arrhythmia. It is of interest to reexamine to what extent this is true.

The diastolic waves are often so recurrent and numerous that it is impossible to account for them under the assumption that the auricle is in a dilated and finely fibrillating state, the condition to which the term auricular fibrillation was first applied. Many electrocardiograms also show definite variations resembling P waves or diastolic wave groups which are interspersed between easily recog-

[1] Lewis, T., Mechanism of the Heart Beat, London, 1911, 198.

nized ventricular complexes. Consequently it has been suggested (Hewlett and Wilson[2]) that in some cases a condition of coarse fibrillation obtains, or that the condition is associated with a state of rapid coordinated contractions designated as auricular tachyrhythmia, or flutter (Jolly and Ritchie[3]). Concerning the diagnostic differentiation between flutter and fibrillation, Ritchie states:

"If polygraph tracings reveal a rhythmic series of large auricular waves in the jugular veins at times when the ventricles are in diastole, the condition is clearly flutter and not fibrillation. On the other hand, if the waves are small, irregular in rhythm, and very rapid, and if at the same time there is little uniformity in the grouping of arterial pulse beats, the auricles are either in fibrillation or in the form of activity representing simultaneous flutter and fibrillation."

Hewlett and Wilson,[2] in 1915, reviewed the experimental relation between fine and coarse fibrillation and comment upon the rarity with which large recurrent diastolic waves are found in venous tracings. They conclude that as a rule clinical fibrillation is of the fine type, but present a case, fully studied by photographic registration, in which distinct diastolic waves, which have no relation to electrocardiogram wavelets, were present. This they interpreted as a condition of coarse fibrillation.

A more comprehensive attempt to establish the extent to which coarse and fine fibrillation occur clinically and to what degree these in turn are associated with flutter is of apparent interest. For these reasons an objective analysis of the jugular and other records taken by photographically recording capsules, was undertaken, and their interpretation harmonized with the case histories and other physical signs present at the time.

Method of Procedure.

Records of the venous pulse were taken simultaneously with the subclavian and radial pulses and with apex beats or electrocardiograms.[4] The superiority of capsules photographically recording the dynamic changes by reflecting beams of light need not be reviewed. The photographic records were obtained by mounting the Frank segment capsules, illuminating lamp, and photokymograph on a

[2] Hewlett, A. W., and Wilson, F. N., *Arch. Int. Med.*, 1915, xv, 786.

[3] Jolly and Ritchie, Auricular Flutter, New York, 1914, 125.

[4] We are indebted to Dr. Alexander Lambert for placing the electrocardiograph in Bellevue Hospital at our service and to Dr. Josephine B. Neal for valuable technical assistance in securing the clinical electrocardiograms.

table which could be wheeled to the bedside (Wiggers[5]). When simultaneous electrocardiograms were taken the table was also aligned with the projection system of a Cambridge model of Einthoven's string galvanometer. The movements of the segment capsule mirrors were thus photographed simultaneously with the shadow of the galvanometer string. Tracings of the jugular and radial pulses were also taken by a Jacquet polygraph for comparison with the photographic records.

Details of the Normal Photographic Curves.

Although the details of the photographic venous pulse have often been described (Edens and Wartensleben,[6] Ohm,[7] Van Zwaluwenburg and Agnew,[8] and Wiggers[9]), our analysis of the photographic tracings in auricular fibrillation will be facilitated by a brief reconsideration of its chief features, especially as related to the photographic apex curves.

The two tracings shown in Fig. 1 give the essential characteristics of normal photographic records from the apex region and supraclavicular fossa. Experience has shown that there is nothing distinctive about the contour of the apex curve, which may in fact be positive or negative according to the position of the patient and placement of the tambour (Wiggers[10]). A detailed description is therefore superfluous. There is, however, one feature of these tracings which makes them valuable, for unless the chest wall is too thick or emphysematous lung overlies the heart, they show clear indications of the heart sounds (S^1 and S^2) which enables a positive determination of the beginning and end of ventricular systole. The photographic venous pulse always shows three essential waves; to these a fourth wave is

[5] Wiggers, C. J., *J. Am. Med. Assn.*, 1915, lxiv, 1305.

[6] Edens, E., and Wartensleben, B., *Deutsch. Arch. klin. Med.*, 1911, civ, 552.

[7] Ohm, R., Venenpuls- und Herzschallregiestierung als Grundlage für die Beurteilung der Mechanischen Arbeitsleistung des Herzens nach eigenen Methoden, Berlin, 1914.

[8] Van Zwaluwenburg, J. G., and Agnew, J. H., Heart, 1911–12, iii, 343.

[9] Wiggers, Modern Aspects of the Circulation in Health and Disease, Philadelphia, 1915, 134; *J. Am. Med. Assn.*, 1915, lxiv, 1485.

[10] Wiggers, Modern Aspects of the Circulation in Health and Disease, Philadelphia, 1915, 151.

sometimes added when the cardiac cycle is long. The waves differ from polygraph curves in that each wave has a distinctive contour and that the relative prominence of the waves is different.

Since the records taken from cases of auricular fibrillation have so little in common with normal curves, it was found desirable to discontinue the commonly accepted terminology (a, c, v waves) introduced by Mackenzie. All records may readily be divided into systolic and diastolic portions. This is usually accomplished by the use of the two sound vibrations previously referred to in the apex tracing. Occasionally these vibrations are transmitted to the neck and superimposed on the venous tracings as well (Fig. 1, S^2). The waves are best designated as presystolic, systolic, and diastolic. In the normal cycle each portion has, as a rule, a single wave. The presystolic wave (P) is associated with auricular systole. The systolic wave (S), occurring a considerable interval after the first sound, is synchronous with the subclavian rise of pressure and is, therefore, due to an arterial impact. The third wave (D_1), commonly called v wave, is entirely diastolic in time, and is probably due to the cessation of stasis, as the tricuspid valve opens at its summit. When the cardiac cycle is long, a second diastolic wave (D_2) is often found. Its significance cannot be positively given at present, but its presence in long cycles should be kept in mind in connection with fibrillation waves.

The following are average durations found for these waves:

	sec.
Presystolic wave (P), or a wave	0.09–0.10
Systolic wave (S), or c wave	0.28–0.32
First diastolic wave (D_1), or v wave	0.15–0.24
Second diastolic wave (D_2), or h wave	0.21–0.22

Types of Waves in Auricular Fibrillation.

Method of Analysis.—The photographic tracings taken from cases of auricular fibrillation were analyzed in the same manner as normal records. The beginning and end of each ventricular systole must first be established. In many instances the first and second sound vibrations of the jugular or apex tracing satisfactorily determine these places; in others, where irregular cardiac action occurs, only a single sound is recorded in the jugular or apex tracing. Since

there is nothing distinctive about heart sound vibrations, it is occasionally impossible in auricular fibrillation to determine whether the vibrations represent the end of a premature but ineffective ventricular systole, a third sound, or a short diastolic murmur. The addition of an electrocardiograph record serves to elucidate these cases since the presence or absence of an R wave at these times differentiates them. Combined simultaneous records of the jugular pulse, apex beat, and electrocardiograms were therefore made in most cases, but as the electrocardiograms taken on bromide paper are not sufficiently clear to reproduce well, they have been omitted in the reproductions.

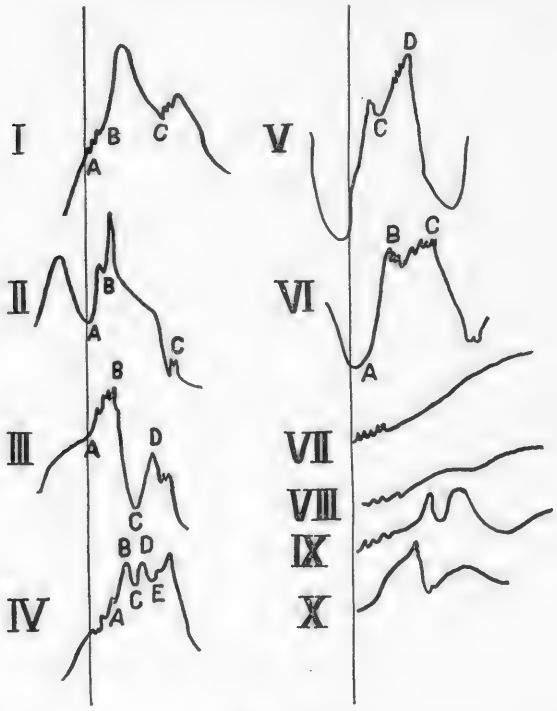
Types of Waves Occurring during Ventricular Systole.

In experimental auricular fibrillation many ventricular systoles occur that are too weak to open the semilunar valves, and hence fail to elevate aortic pressure, or elevate it so slightly that a pulse wave is not propagated to the more peripheral arteries (Wiggers[11]). The frequent occurrence of a pulse deficit is evidence that this also occurs in clinical fibrillation. The ventricular systoles may, therefore, be divided into effective and ineffective groups. Effective systoles may be recognized by the presence of a corresponding pulse wave in the subclavian or radial tracings. Ineffective systoles are often indicated by a single sound in the apex curve, but more certainly by the presence of a ventricular complex in the electrocardiogram without a corresponding arterial wave.

Types of Waves during Effective Systoles.—The different types of waves occurring during effective systoles are illustrated by the actual records shown in Figs. 2 to 9, but for a description, reference may be made to the transferred waves shown in the curves of Text-fig. 1.

The systolic waves may resemble typical impact waves such as are found in normal pulses. As in Type I, the rise is usually preceded by a short isometric period (A–B), during which vibrations of the first sound are discernible. The wave terminates in the vibrations of the second sound at C. The impact wave may, as shown in Type II, rise and fall sharply, which is also a frequent nor-

[11] Wiggers, *Arch. Int. Med.*, 1915, xv, 77.



TEXT-FIG. 1. I to VI represent different types of systolic waves occurring in auricular fibrillation. VII to X represent diastolic waves. Transcribed from the original curves and reduced about one-third.

Type I. Normal and intensified impact wave, often the only characteristic feature, and sometimes interpreted as the ventricular type of the venous pulse.

Type II. Peaked impact wave followed by systolic drop. It occurs when the tambour is pressed lightly and intravenous pressure is low during systole.

Type III. Intra-auricular type of venous pulse. It most nearly resembles the intra-auricular pressure curve and is only recorded when the arterial impact is feeble or absent.

Type IV. Double systolic waves both completed during systole. Probably due to tug or position change of contracting ventricle.

Type V. Systolic impact and stasis wave. Double systolic wave differing from Type IV in that the summit of the second wave terminated at the end of systole.

Type VI. Regurgitation wave due to tricuspid insufficiency.

mal variation (Fig. 1) when the tambour is very lightly applied. A direct impact may be almost or completely absent, as in Type III, in which a marked fall similar to that observed within the auricle, is the predominant feature. This is followed by a systolic rise (C–D), which also resembles the auricular pressure curve. This probably as nearly corresponds to a pure venous curve as it is possible to obtain and probably occurs only when the arterial waves are not vigorous enough to superimpose an arterial impact on the tambour record. A fourth type of wave is shown in Type IV where an impact oscillation (A–B–C) is followed by a second systolic oscillation (C–D–E). Its occurrence before the end of systole cannot be questioned, as the vibrations of the second sound occur after it terminates. Its significance cannot be fully explained. The possibilities that it may represent a tricuspid regurgitation late in systole, or an auricular stasis, do not entirely accord with the fact that it falls before diastole begins. The possible explanations to be considered are: (*a*) a ventricular position change which modifies auricular pressure and hence the jugular curve; and (*b*) a tug of the contracting ventricle on the superior vena cava. This is made more probable by the fact that waves of this type were particularly marked in two of our cases, both of which had definite signs of adhesive mediastino-pericarditis, and pericardial adhesions may well result in an unusual traction upon the superior attachment of the sac. A second type of double systolic wave is shown as Type V. This differs from Type IV in that the summit of the second wave terminates at the beginning of diastole.

What may be designated as regurgitation waves were also found. These waves have a distinctive contour—entirely different from the rounded ventricular waves described by Mackenzie and others in polygraph curves. These waves (Type VI) resemble waves found within the auricle when a relative insufficiency occurs during fibrillation of the auricles. Early in systole they rise sharply (A–B), then gradually rise further or remain sustained until the onset of diastole (C). Particularly characteristic are small undulations or vibrations superimposed on their summits which represent murmur vibrations transmitted to the supraclavicular region and generally audible there.

Waves Occurring during Ineffective Systoles.—During ineffective systoles the venous pulse may show nothing except a gradual stasis (Type VII) terminating at the end of systole; or the isometric period may be followed by a double vibration similar to, but smaller than those sometimes found in effective systoles (Type VIII). These waves may occasionally be shorter and more prominent (Type IX), and evidence of a small regurgitant wave is sometimes found (Type X).

Diastolic Waves.

The diastolic waves occurring during auricular fibrillation may be classed as large and small. The large oscillations are frequently found just before the systolic wave. In contour and duration they cannot be distinguished from normal presystolic (a) waves. Similar waves are also found during mid-diastole as shown at D in Fig. 9, and when this event is long they may occur in numbers. They apparently correspond to the waves found in Hewlett and Wilson's reported case. Instead of being a rare event, however, we find that they are a very common occurrence in photographically recorded venous pulses from cases of auricular fibrillation.

Three possible explanations are suggested for these waves, but it is not possible to decide between them on the basis of clinical experiment alone. It is conceivable that they may be produced by a coordinated mechanical contraction of a considerable portion of the auricular musculature which is sufficient to inaugurate a pressure wave within the auricle. According to this view, we must assume that the incoordinated fractionate contractions[12] of individual units of cardiac tissue happen to be so related that from time to time their interference produces an actual shortening of a certain section of the auricle. If this were the case we would expect simultaneous waves in the electrocardiogram which resemble normal P waves. Such waves are occasionally to be found, but the synchronism is by no means frequent.

It is possible that these waves indicate incoordinate coarse fibrillations of the auricle, as suggested by Hewlett and Wilson, since

[12] The term "fractionate contraction" has been defined by one of us as the interval that any unit of auricular tissue continues to shorten (*Am. J. Physiol.*, 1916, xl, 222).

they are irregular in size and bear no relation to smaller variations of the electrocardiogram. If this interpretation is true, then coarse fibrillations are far more common clinically than is generally believed.

Inefficient contractions of the ventricle might produce such waves. The possibility has been shown in animal experiments by one of us, and indeed was at the same time considered the most probable cause of these waves. Though virtually diastolic waves, they would really be due to unrecognized systoles. If this were true the electrocardiograms would show distinct ventricular complexes synchronous with them. A careful study of our records shows, however, that such inefficient systoles are rarely present in clinical fibrillation, and that very few such waves can be accounted for upon this basis.

The resemblance of the presystolic waves found in fibrillation to the second diastolic wave of the normal venous pulse has led Lewis[13] and others to attribute it, as has been done in the normal pulse, to a floating together of the tricuspid valves. Neither in the normal or fibrillating pulses does this interpretation appeal to us. The facts (*a*) that the wave is not associated with the third sound in cases where the latter has been recorded (Einthoven,[14] Eyster[15]), (*b*) that the valves do not come together sharply at the beginning of diastole, but apparently gradually float together throughout diastole (Dean[16]), (*c*) that, on dynamic principles, their closure is probably caused by a pressure difference rather than a wave of pressure within the auricle, all lead one to question such an origin in normal pulses. The fact that several such waves occur during long diastolic intervals in fibrillation would necessitate the assumption that the valves move into their position of closure several times, while an isolated presystolic wave occurring at the end of a long diastole would necessitate the assumption that in these beats the closure was, for some unaccountable reason, greatly delayed. That these waves are similar to the late diastolic waves normally found in long cycles cannot be denied; that either owes its origin to a valve movement seems improbable.

[13] Lewis, Mechanism of the Heart Beat, London, 1911, 204.
[14] Einthoven, W., *Arch. ges. Physiol.*, 1907, cxx, 31.
[15] Eyster, J. A. E., *J. Exp. Med.*, 1911, xiv, 594.
[16] Dean, A. L., Jr., *Am. J. Physiol.*, 1916, xl, 206.

They may be due to a position change of the relaxing or filling ventricle which exerts a pressure traction upon the auricles or large veins or perhaps causes a variation of intrathoracic pressure which might modify the influx and efflux of venous blood in the extrathoracic veins.

Records of Representative Cases.

As it is manifestly impossible to reproduce the many hundred yards of records taken from a total of 25 cases in the Second Medical Division of Bellevue Hospital, a description of a few short segments from representative cases must suffice for presentation. In this selection have been included (1) cases in which systolic waves predominate including (*a*) slow rhythms and (*b*) rapid rhythms, and (2) cases in which diastolic waves predominate (slow rhythms).

Case H, 19.—Male, aged 51 years. Rheumatic heart; no murmurs; compensating. Chronic nephritis. Systolic blood pressure, 165. (Fig. 2.)

The photographic record shows that systolic waves are predominant at the jugular region. Numerous attempts to obtain other venous waves of considerable size proved futile. Each systolic wave is nothing more than a transmitted arterial pulse. This is the characteristic record found in cases of auricular fibrillation accompanied by strong cardiac action and elevated blood pressure. The details of the central arterial pulse are readily discerned: 1–2, the initial vibrations; 3, the primary peak; and 4, the incisura with after vibrations. Their irregularity is the only conspicuous difference from normal arterial curves.

If we glance at the supraclavicular record taken by a Jacquet polygraph (Fig. 3) by way of comparison, it shows a series of systolic waves in every way typical of the ventricular type of venous pulse and referred to tricuspid regurgitation. It is clear that the deforming action of tambour levers may distort a perfectly normal arterial impact curve into the semblance of a regurgitation wave.

During the longer diastoles of Fig. 2 small waves occur (d–d) having a period of 0.076 to 0.128 of a second. Similar corresponding wavelets were also found in the apex curves.

Case G, 6 and H, 8.—Male, aged 44 years. Sclerotic heart; no murmurs; non-compensating. Chronic nephritis. Blood pressure, 220–150. (Fig. 4.)

This case showed in succession a variety of systolic wave types and in addition was characterized by numerous large diastolic waves during a certain period of his hospital stay. The cycles in this segment of record are numbered from 3 to 7 to facilitate description. Wave 3 represents a normal impact wave. The isometric period (a–b) is filled with fused preliminary vibrations after which the impact (b–c–d) occurs. Cycle 7 resembles this wave, but the systolic decline is sharper, placing it under Type II. Cycle 4 shows a bifurcated systolic wave (Type IV) which, to judge from the smaller amplitude of the corresponding radial beat, was produced by a less vigorous systole. Such smooth double vibrations were often found to accompany weak systoles in animal experiments previously reported by one of us.[11] Cycle 5 would probably have shown a similar double wave had not a weak systole occurred at 6 and cut short the second vibration. After the first elevation of Cycle 6, the curve continues to rise owing to the stasis of blood in the auricle which had not been allowed to empty because of the practical absence of preceding diastole. This double vibration due to an ineffective systole corresponds to Type IX.

During diastole a series of large waves (D_1, D_2, and D_3) occurs. While the third oscillation, D_3, is at its crest, Systole 4 occurs. Without a careful analysis of the interval of systole, these systolic waves following might be attributed to the same cause as the diastolic waves D_1, D_2, and D_3 and held to indicate a rhythmic coarse contraction of the auricle.

Attention should be called to the presystolic wave, P, which precedes Systole 7. In time and general appearance it resembles a normal presystolic wave, or a wave due to a coordinated auricular activity. These waves occur so frequently that to say presystolic waves are absent in auricular fibrillation compels one to ignore many such waves not to be distinguished from normal a waves.

To summarize, this record shows in rapid sequence a variety of systolic waves when ventricular activity is irregular in force, and the presence of large diastolic waves having a rate of 5.5 per second, or 330 per minute.

Case H, 18.—Male, aged 51 years. Sclerotic heart; rate 140–150 per minute; relative mitral insufficiency. General anasarca. Deep, somewhat dyspneic breathing. (Fig. 5.)

When the heart rate is rapid the venous pulse is composed almost entirely of systolic waves. When, in addition, breathing is deep, they are not only superimposed upon the respiratory variations, but their contour changes with inspiration and expiration, as in this record. During inspiration the individual waves are small and scarcely recognizable (*e.g.*, 2, 3, and 9). During expiration (Waves 1, 5, and 6) the waves are larger and have a distinctive contour which places them under Type II. The correspondence of the R peaks with each radial pulsation shows that each systole, even at that rapid rate, was effective.

Case H, 17.—Male, aged 35 years. Dilated, rheumatic heart; mitral regurgitation (Fig. 6); left-sided hypertrophy shown by electrocardiogram. A harsh systolic murmur was heard over the apex of the heart and in the right supraclavicular fossa.

The venous tracing shows a series of prominent regurgitant waves, the characteristic features of which are a rapid rise during the onset of systole ending in a sustained ascending or descending plateau upon which a series of vibrations, probably resulting from the concurring murmur, are superimposed. In contour they correspond to intra-auricular waves found in experimental animals which have tricuspid regurgitation. The onset of systole as shown by the R wave of the accompanying electrocardiogram (not reproduced) is marked at S in each case. It is apparent that their exact contour depends to some extent upon the state of venous pressure at the onset of systole. Beat I represents a typical unaltered regurgitation curve. In Waves IV and V the onset of systole occurred at the summit of a diastolic stasis rise (S) and consequently the main rise of the wave occurred before the onset of systole. This is a frequent occurrence when the heart rate is rapid.

Case H, 11.—Male, aged 40 years. Rheumatic heart; very large. Systolic murmur over aortic area transmitted to the neck; diastolic murmur over sternum transmitted to the apex; systolic murmur at the apex transmitted to the axilla. (Fig. 7).

The record of Fig. 7 was taken on February 19, 1915. The pulse rate averaged 150 per minute. The venous pulse is chiefly made up of waves indicating a dilated right heart. Two types are prominent. Those marked Type V show after a primary systole wave, which is probably an impact, a considerable stasis rise. Several waves, owing to weaker systoles, show little of the first impact wave and consist essentially of the early systolic fall and subsequent rise. These are the waves that resemble the intra-auricular pressure curves (Type III). Between these waves occur typical regurgitant waves, which rise rapidly and remain sustained with numerous vibrations superimposed throughout systole (Type VI).

Another record shown as Fig. 8 was taken on March 1, 1915, after rest and digitalis treatment had slowed the pulse to an average of 86 per minute. This lengthened the diastolic interval which is consequently filled with many diastolic waves (d) varying considerably in amplitude and period. There are no corresponding waves found in the electrocardiogram record. Evidence of regurgitation waves is practically absent.

Case H, 1.—Male, aged 38 years. Infective endocarditis and mediastino-pericarditis; double mitral lesion; coarse systolic thrill over precordium (Fig. 9). Upper curve, supraclavicular venous pulse; lower curve, apex tracing.

During systole two distinct waves occur in the apex tracing, one (1–2–3) during the early half of systole, the second (3–4–5) during the latter half. Superimposed upon these waves occurs a series of finer waves extending throughout systole, as can be seen from the irregular width of the ascending line (3–4–5) They represent the first sound vibrations together with the systolic murmur. Systole ends with the few vibrations at S^2.

Each systole produces in the jugular pulse two waves, 1–2–3 and 3–4–5, which, allowing for delay, correspond exactly to the apex waves. The probability that this double systolic wave (Type IV) is due to a position change or tug of the ventricle has been previously discussed.

Early in diastole of each cycle occurs a wave D similar to that sometimes found in the normal curve. When the cycles are long this wave may precede systole and simulate a presystolic wave, as in the third cycle shown.

TABLE I.

Relative Frequency of Different Types of Waves.

Case No.	Age.	Clinical diagnosis.	Systolic waves.					Diastolic waves.
			Normal impacts. Types I and II.	Intra-auricular curves, feeble ventricular action. Type III.	Systolic tug or position change. Type IV.	Stasis curves. Type V.	Regurgitation curves. Type VI.	
	yrs.							
G, 1	32	Rheumatic heart; mitral stenosis.	All.					Few fine in long diastoles. Period short.
G, 2	21	Rheumatic heart; mitral regurgitation.					All.	Many coarse. Period irregular.
G, 5	62	Sclerotic heart; relative mitral regurgitation.				All.		Few coarse.
G, 6-H, 8	44	Sclerotic heart; no murmurs; chronic nephritis. Blood pressure 220–150.	Many.		Few.			Many coarse. Frequency, 330 per min.
G, 7	54	Rheumatic heart; double mitral lesion.	All.					None.
G, 9	26	Rheumatic heart; double mitral lesion.	Many.			Few.		Many fine in long diastoles. About 750 per min.
G, 10	42	Rheumatic heart; mitral regurgitation.			All.			None.
G, 11	38	Rheumatic heart; mitral regurgitation.	All.					Many coarse.
G, 14	26	Rheumatic heart; mitral regurgitation.	"					None.
H, 1	38	Infective endocarditis and mediastino-pericarditis; double mitral lesion.	Many.		Many.			Many fine. Frequency 460 per min.
H, 5	48	Postpneumonic mediastino-pericarditis; dilated heart; no murmurs.	"		"			Occasional presystolic.
H, 7	61	Sclerotic heart; dilated; no murmurs.	All.					Many fine.
H, 9	36	Rheumatic heart; mitral regurgitation.	"					None.
H, 10	30	*Streptococcus viridans* polyserositis; dilated heart; relative mitral regurgitation.	"					Occasional coarse. Period from 0.076 to 0.128 sec.

TABLE I—*Continued.*

Case No.	Age.	Clinical diagnosis.	Systolic waves.					Diastolic waves.
			Normal impacts. Types I and II.	Intra-auricular curves, feeble ventricular action. Type III.	Systolic tug or position change. Type IV.	Stasis curves. Type V.	Regurgitation curves. Type VI.	
	yrs.							
H, 11	40	Rheumatic heart; mitral regurgitation; aortic regurgitation and roughening.	Few.	Few.		Many.	Many.	Few coarse. Frequency, 300 per min.
H, 12	48	Rheumatic heart; double mitral lesion.	All.					Few presystolic.
H, 13	37	Rheumatic heart; mitral regurgitation.	"					Many fine.
H, 15	27	Rheumatic heart; double mitral lesion.	Many.			Few.	Few.	" " Frequency, 450 per min.
H, 16	24	Rheumatic heart; double mitral lesion; Graves' disease.	"			"		Few fine. Period from 0.07 to 0.10 sec.
H, 17	35	Rheumatic heart; mitral regurgitation.	"				Many.	Few fine.
H, 18	51	Sclerotic heart; relative mitral regurgitation.	All.					None.
H, 19	51	Rheumatic heart; no murmurs; chronic nephritis.	Many.			"		Few coarse in long diastoles; more as ventricle slowed. Period from 0.096 to 0.128 sec.
H, 20	38	Rheumatic heart; double mitral lesion.	All.					Few fine.
H, 21	55	Sclerotic heart; relative mitral regurgitation.	Few.			"	"	" "
H, 23	34	Rheumatic heart; double mitral lesion.	"	Many.		"		Many fine. Period from 0.066 to 0.116 sec.

In the apex curve as well as the jugular curve occur small wavelets (d–d) whose period averages 0.13 to 1.1 seconds. Their rate averages about 460 per minute.

This case, illustrating a fibrillating auricle with slow ventricular

rhythm, shows in the jugular a typical double systolic wave, a normal diastolic variation, and, in addition, small periodic diastolic waves.

The electrocardiogram showed similar diastolic waves of considerable amplitude, which did not correspond definitely with the waves in the phlebogram.

The relative frequency with which the various types of waves occurred in our cases is shown in Table I.

By far the most frequent form of systolic wave is the normal impact wave (Types I and II), which was present in all but three cases. They often constitute the most conspicuous, and in certain cases (twelve of our series) the only characteristic feature of the venous pulse curves. They may resemble each other from one cycle to another, which is usually the case when the heart is slow and comparatively regular, or they may show rhythmical variations in size with respiration, especially when the heart is rapid yet fairly regular, or when moderate dyspnea is present. They may vary from beat to beat both in contour and amplitude, these being determined, as has been previously analyzed by one of us,[11] by (*a*) the vigor of ventricular systole, (*b*) the height of aortic pressure at the onset of systole, (*c*) the duration of the previous diastolic interval, and (*d*) the pressure of the recording tambour.

When the cardiac contraction is particularly vigorous the systolic wave may resemble a typical central arterial pulse terminating in a deep incisure. The important observation was made that when this occurs the venous pulse recorded by the polygraph is unusually distorted and often resembles the ventricular waves of Mackenzie, which are supposed to indicate regurgitation. So called ventricular waves of polygraph curves may thus be produced by perfectly normal arterial impacts and are not indicative of tricuspid regurgitation; on the contrary, they may signify exceptional heart action.

Next in frequency is the stasis curve (Type V), being found in eight cases. In only one instance were they the only curves present, and, as might be anticipated, they were most frequent when the heart was incompetent and venous stasis was evident in the pulmonary or portal circulations.

The type of wave which we attribute to a systolic tug or position change of the heart (Type IV) was present in four cases. In two of

these cases well marked signs of pericardial adhesions were noted, while in another case though no physical signs of the condition were recorded, the rheumatic origin of the heart lesions makes its presence not improbable. In the fourth instance pericardial adhesions were not suspected.

Intra-auricular pressure waves (Type III) were present in only two cases. Regurgitation waves (Type VI) were present in five cases, and in one of these they disappeared during the course of hospital observation.

This observation discredits the idea that tricuspid regurgitation is a frequent accompaniment of auricular fibrillation.

SUMMARY.

While auricular fibrillation is easily recognized by arterial and jugular tracings or by electrocardiograms, it is not possible to interpret all the waves found in these records.

The venous tracings may in general be placed in one of two classes: (*a*) those in which prominent systolic waves predominate or occur alone; and (*b*) those in which large or small diastolic waves occur occasionally, in groups, or in a continued series averaging 250 to 500 per minute. The prominent systolic waves have generally been attributed to tricuspid regurgitation, the inference being that tricuspid regurgitation is a common state in auricular fibrillation. The diastolic waves are so numerous and so many electrocardiograms show diastolic waves that it is impossible to account for them on the assumption that the auricle is in a dilated and finely fibrillating state. It has been suggested that in these instances a condition of coarse fibrillation, which is closely allied to auricular flutter, obtains. The systolic and diastolic waves of the venous pulse of twenty-five clinical cases of auricular fibrillation, recorded by photographic methods, were studied.

Six types of systolic waves (Text-Fig. 1) were found: (1) an intensified impact wave, the most common and often the only characteristic feature, indicating vigorous ventricular action; (2) a peaked impact followed by a rapid systolic drop due to light pressure of the tambour; (3) the intra-auricular type of systolic variation, so called from its resemblance to intra-auricular pressure

curves found in animals, occurring in clinical cases only when ventricular systole is weak; (4) double systolic waves, attributed to a systolic tug of the ventricle on the auricles and large veins; (5) a systolic impact followed by a stasis wave, present when intravenous pressure is high; (6) a regurgitation wave composed of a steep rise continued into a systolic plateau with murmur vibrations superimposed.

Our study showed (1) that tricuspid regurgitation, as indicated by the presence of regurgitation waves, is a rare accompaniment of auricular fibrillation; and (2) that the contrary opinion, arrived at by the frequent presence in polygraph tracings of ventricular types of waves, is due to the fact that the contour of intensified impact waves is distorted by polygraph levers so that they simulate regurgitation waves.

Recurrent diastolic waves were frequently present in our records. Their relative size depended, to a considerable extent, on the pressure of the tambour. There was no constant relation to similar waves in the recorded electrocardiogram, nor is it proven that they are indicative of a coarse type of fibrillation or an associated flutter.

EXPLANATION OF PLATES.

PLATE 1.

FIG. 1. Curve showing the character of the normal apex and venous pulses photographically recorded. Reduced nine-tenths.

FIG. 2. Curves of supraclavicular and radial pulses from a case of auricular fibrillation in which prominent arterial impact waves predominate. During diastole small waves (d–d) sometimes recurred. Reduced six-tenths.

FIG. 3. Venous tracing taken by a Jacquet polygraph from the same case as Fig. 2, showing the so called ventricular type of the venous pulse.

PLATE 2.

FIG. 4. Curves of supraclavicular and radial pulses from a case of auricular fibrillation showing the variety of systolic waves and numerous large diastolic waves. Reduced eight-tenths.

FIG. 5. Supraclavicular and radial pulses in auricular fibrillation with rapid heart rate and deep breathing. The lines correspond to the rise of the R waves of the electrocardiogram simultaneously recorded but not reproduced. Reduced nine-tenths.

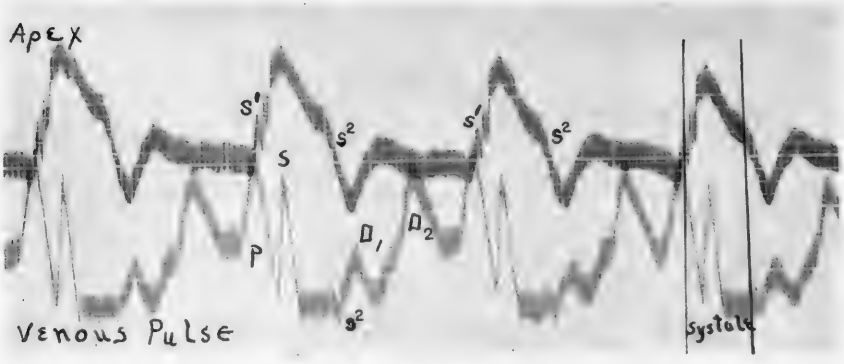


FIG. 1.

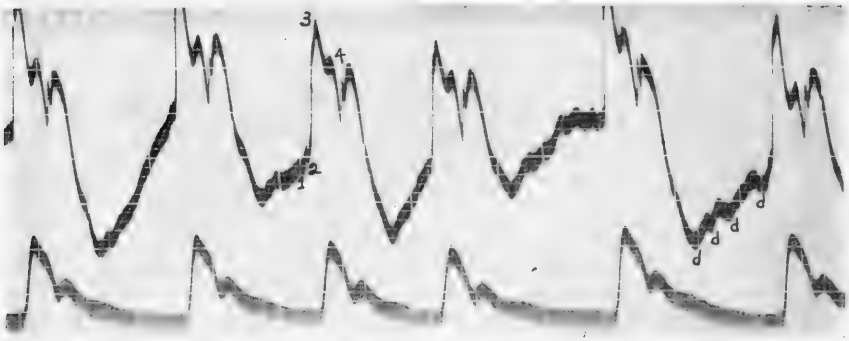


FIG. 2.

FIG. 3.

(Niles and Wiggers: Venous Pulse in Auricular Fibrillation.)

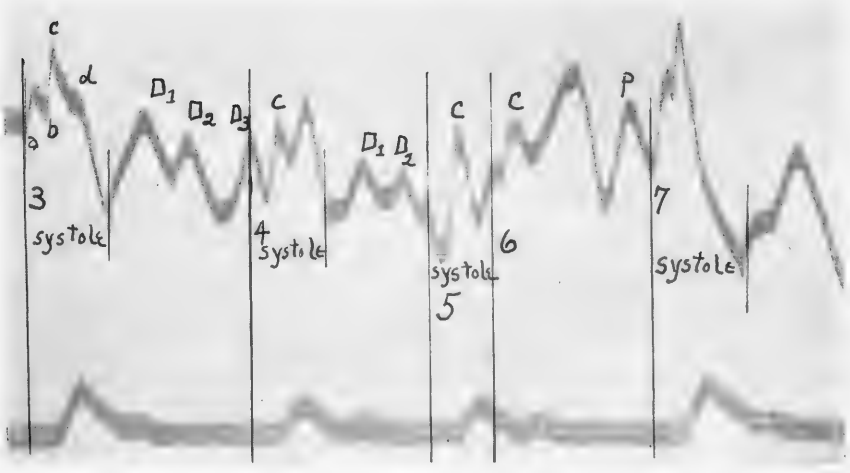

c
a
b
d
D1
D2
D3
c
D1
D2
c
c
P
3
systole
4
systole
systole
5
6
7
systole

FIG. 4.

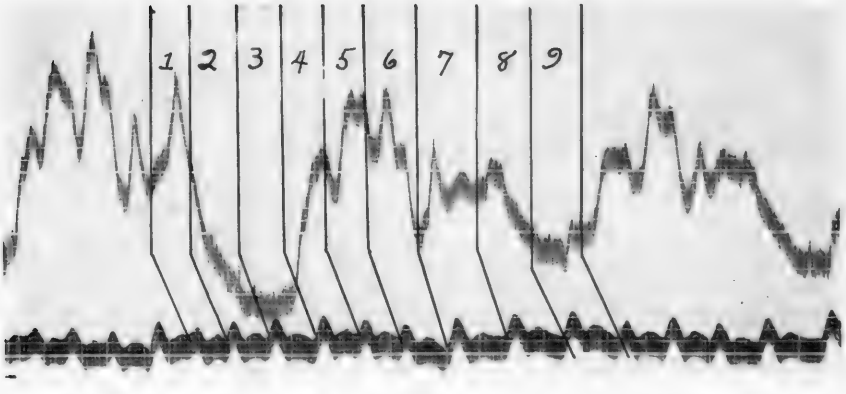

1
2
3
4
5
6
7
8
9

FIG. 5.

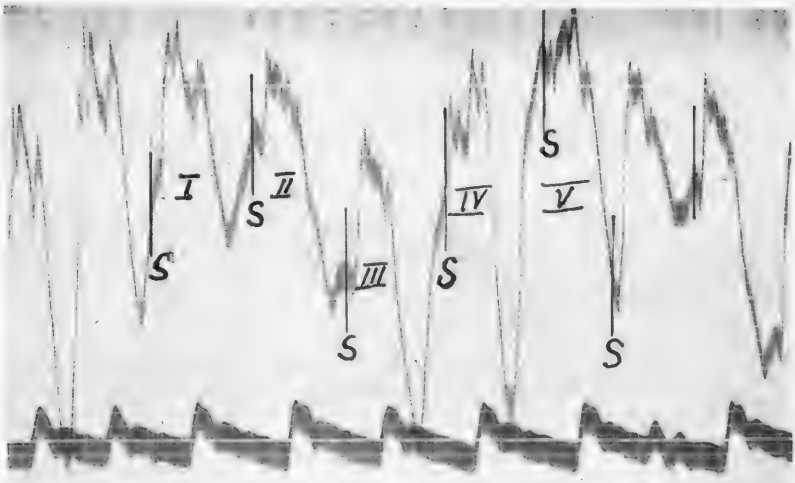


FIG. 6.

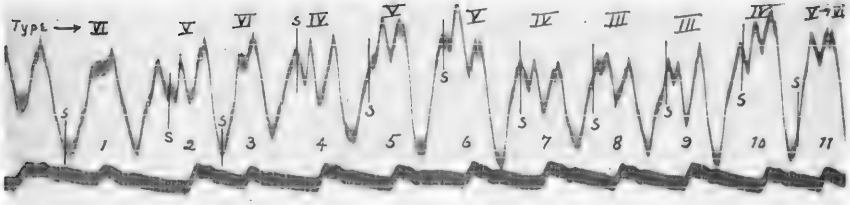


FIG. 7.

(Niles and Wiggers: Venous Pulse in Auricular Fibrillation.)

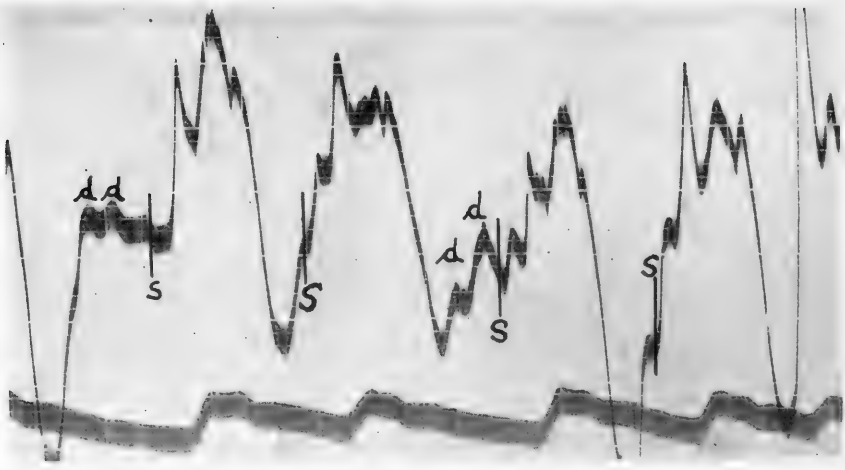


FIG. 8.

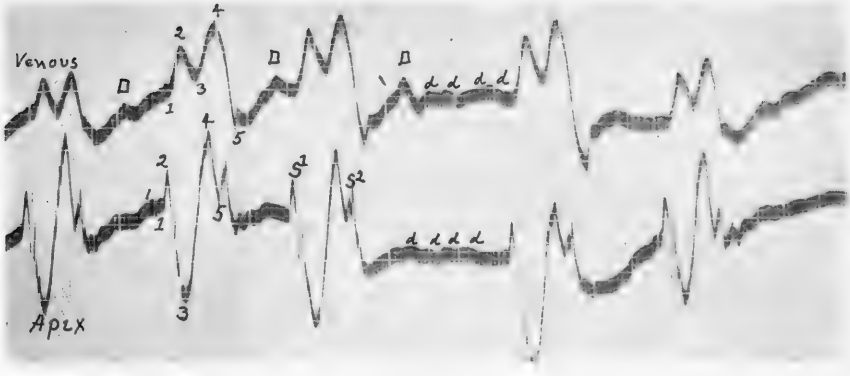


FIG. 9.

(Niles and Wiggers: Venous Pulse in Auricular Fibrillation)

PLATE 3.

FIG. 6. Supraclavicular and radial pulses in auricular fibrillation. The former shows regurgitant waves almost exclusively. Lines are drawn to correspond to R waves of the electrocardiogram simultaneously recorded, but not reproduced.

FIG. 7. Supraclavicular and radial pulses from a case showing a gradation of systolic types of waves. Marked according to the scheme shown in Text-fig. 1. Reduced one-half.

PLATE 4.

FIG. 8. Venous and radial pulses from the same case as Fig. 7 after rest and digitalis medication. The absence of regurgitation waves and the presence of diastolic waves is shown. Reduced nine-tenths.

FIG. 9. Venous and apex curves, showing similarity of waves (p. 13). Reduced about one-half.

THE SIGNIFICANCE OF THE DIASTOLIC WAVES OF THE VENOUS PULSE IN AURICULAR FIBRILLATION.

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Plates 5 to 7.

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General Outline of Plan of Study and Results.

In a previous study of the photographic venous tracings taken from clinical cases of fibrillation,[1] it was shown that the types of waves occurring during systole could be satisfactorily harmonized with and explained by photographic tracings previously obtained from animal experiments. The diastolic waves, however, could be given several different interpretations. This phase of the subject was, therefore, submitted to further experimental investigation.

The plan of study resolved itself into several phases. In the first place, it was desirable to determine whether large diastolic waves in the jugular tracings and in the electrocardiograms of many cases are diagnostic of a state of coarse fibrillation or associated flutter, or whether they are also present during fine fibrillation. Anticipating somewhat the results presented later, it was found that although coarse fibrillation was accompanied by somewhat larger diastolic waves, vibrations of considerable amplitude could be present during a state of fine fibrillation. In the electrocardiograms the diastolic waves were sometimes larger during the state of fine fibrillation.

The second phase of the experiments concerned itself with the cause of these diastolic variations. It at once became important to establish whether they represented pressure variations from the right auricle. To determine this, the right intra-auricular pressure was

[1] Niles, W. L., and Wiggers, C. J., *J. Exp. Med.*, 1917, xxv, 1.

recorded by introducing into the auricle by way of the azygos veins and superior vena cava, a sensitive venous optical manometer (Wiggers[2]). Again anticipating, it was found that with very few exceptions the true diastolic waves[3] were not accompanied by similar variations of intra-auricular pressure, even when extremely sensitive manometers were used.

By elimination it is impossible to ascribe the jugular waves to any other factor than a traction of some portion of the heart upon the veins leading to the lower cervical region. By attaching threads connected with delicate tambour systems to different portions of the veins, and by studying the accurately recorded myograms of the auricle, it was found that during fibrillation a distinct periodic traction is exerted upon the veins and that this corresponds in rhythm with the diastolic waves of the venous pulse.

What type of cardiac action is able to affect the extrathoracic veins so as to alter their lumen and pressure without acting upon the intra-auricular pressure? Previous experimentation had shown that position changes of the contracting and relaxing ventricles are capable of exerting a traction upon the auricle. A comparison of these traction oscillations of the auricular myogram with the traction curves of the large veins and with the venous waves, led to the conclusion that the position changes of the relaxing ventricle could produce the large oscillations, which accompany fine fibrillation. This, however, is not the only mechanism capable of producing diastolic waves during coarse fibrillation. It was shown by myographic curves and auricular electrograms that the irregularly spreading excitation waves strike different areas of the auricle in such a way that they undergo irregular mechanical contractions. These contractions are apparently also able to exert a traction on the veins sufficient to cause diastolic waves in the venous pulse. These coarse contractions bear no relation to the electrical waves and the latter are therefore not of differential value between coarse and fine fibrillation.

[2] Wiggers, C. J., *Arch. Int. Med.*, 1915, xv, 77.

[3] Double rounded waves, which are systolic in time, may, as previously pointed out, be mistaken for diastolic, unless accurate evidence of the presence of a systole is recorded. Such waves are accompanied by intra-auricular pressure variations.

Having thus outlined the plan and object of the experiments and anticipated the results in a general way, it is desirable to present more in detail the precise procedures and results from which the conclusions are derived.

Experimental Procedures.

Dogs anesthetized with morphine and chloretone were used. The external jugular vein was dissected low down in the neck. Over the venous pulsation a small tambour (2 cm. in diameter) covered with light rubber was lightly pressed and the volume changes of the vein were communicated to Frank's segment capsules as in clinical cases. Electrocardiograms were recorded from the right fore leg and left hind leg (Lead II) by Dr. Williams' American model of Einthoven's string galvanometer.

The thorax was opened and records of the intra-auricular pressure were taken by the type of optical manometer described elsewhere (Wiggers[2]). This instrument was, in a number of experiments, made exceedingly sensitive with the hope of detecting the slightest variation of intra-auricular pressure. So used, these manometers unfortunately pick up vibrations of the animal and of the building which appear as small rhythmic oscillations, and must, of course, be discounted in the analysis of records.

To various points on the right auricle were stitched several miniature myocardiographs recently described (Wiggers[4]). These instruments recorded, through optical capsules, the approximation and recession of the points to which they were attached.

Auricular fibrillation was induced by applying a moderate tetanizing current by a pair of ordinary platinum electrodes, or by sending the current through pin electrodes inserted into widely separated points on the auricle. The latter mode of stimulation more frequently produced a fine fibrillary movement; the former, a coarser fibrillation. We also attempted to produce fine fibrillation by simultaneously stimulating the right vagus, as reported by Robinson.[5]

When an electrocardiogram was being taken, the electrical variations of the stimulating current naturally spread so as to affect the

[4] Wiggers, *Am. J. Physiol.*, 1916, xl, 218.

[5] Robinson, G. C., *J. Exp. Med.*, 1913, xvii, 429.

galvanometer leads and hence are indicated in these records. For this reason the fibrillating movements persisting immediately after cessation of stimulation were recorded whenever possible.

During fibrillation of the auricle the ventricular rhythm becomes irregular and the rate exceedingly rapid. This condition does not correspond to the majority of cases seen clinically after treatment where the beat is slow. A corresponding slowing was experimentally produced in a number of ways, the two most successful means of producing a prompt slowing being the stimulation of the left vagus and the use of pituitrin.

Analysis of Results.

Anyone who is familiar with the technical limitations of optical experiments realizes that it is not feasible to record more than three or four circulatory records at a single time. Hence various combinations of tracings were taken in a total of 18 experiments. In this way the different events were related and the results many times duplicated. In analyzing the results it is not possible to proceed in a manner as methodical as that used in the general outline of results, because each record brings out isolated points which make the basis of the facts. It is proposed, however, in the selected records appended to discuss each phase of the investigation as outlined.

Fig. 1 is a section of a record taken on June 16, 1916. The intra-auricular pressure, the venous pulse, and auricular myogram from two points on the auricle 16 mm. distant were recorded. They are indicated on the record.

The record starts while the auricle is in fine fibrillation caused by the continued passage of a moderate tetanizing current. This condition continued during the period marked A–B. Upon cessation of stimulation the auricle lapsed into a state of coarse fibrillation, extending through the interval marked B–C. This was followed by a condition of regular auricular and ventricular beats.

The myogram record is interesting, first as showing the mode of cessation of auricular fibrillation in the dog. It is at once apparent that fine fibrillation (A–B) causes exceedingly small variations in the myogram curve, which cannot be recorded at all by ponderable

levers. This means that the combined effects of the separate fibrillary movements spreading irregularly over the auricle do not by interference or summation act to produce a mechanical shortening of auricular tissue. When stimulation is discontinued the auricle of dogs frequently regains its normal rhythm rapidly. Before this occurs, however, the auricle lapses into a condition of coarse fibrillation. During this interval (B–C) the auricular myogram shows definite waves indicating that distinct mechanical shortening is taking place. Within the interval of 1 second six such waves occur, making their rate 360 per minute. During this period, moreover, two large beats (M, N) occur which resemble the myograms of coordinated contractions. An examination of the intra-auricular pressure curve, taken with a very sensitive manometer (1 mm. = 0.8 mm. saline), shows that after discounting the fine periodic vibrations (1, 2, 3) due to extraneous vibrations and the waves due to ventricular systole (sys), there are no waves during either fine or coarse fibrillation. The first auricular attempt at a definite contraction (M) is also accompanied by no marked rise of intra-auricular pressure. The second contraction (N), on the contrary, is accompanied by a definite wave (N′). Time comparisons show that the rise of pressure corresponds absolutely to the onset of the myogram contraction. The onset of real coordinated contractions (O, P) is indicated by their characteristic contour (Wiggers[4]). These contractions are preceded for the first time by the pressure rise, as is normally the case.

The second point of interest lies in the relation of the diastolic waves of the venous pulse to the auricular myogram and intra-auricular pressure variations. It will be seen that between the waves accompanying ventricular systole (sys) are definite diastolic waves (a) occurring at the rate of 6 to 8 per second. Although somewhat smaller, they are present during coarse as well as fine fibrillation. During the former condition they appear without any evidence of mechanical shortening in the auricular myogram. During the latter condition, as shown by the lines sketched, they follow a short interval after the smaller oscillations in the myogram.

A careful consideration of the records brings out the following points. (1) When the auricle passes out of a condition of fine fibrillation, it first passes through a stage of coarse fibrillation. During

this stage definite mechanical contractions gradually appear and these finally give way to normal coordinated contractions. (2) Diastolic waves of the venous pulse may occur during the state of fine as well as coarse fibrillation. In the latter condition they are, however, generally larger in amplitude, and correspond to an actual mechanical shortening of the auricle. (3) The diastolic waves of the venous pulse are not due to transmitted auricular pressure variations during fibrillation. Such variations occur only when the auricle gives coordinated contractions.

The records shown as Fig. 2 are from an experiment on March 8, 1916. The upper record shows the auricular myogram taken by a miniature myocardiograph and recorded by a segment capsule more sensitive than that used in the records of Fig. 1. The electrocardiogram (Lead II) is recorded below with time divisions of 0.05 of a second.

The records are typical as showing the relations between the electrical variations and accurately recorded mechanical changes in the auricle during fine and coarse fibrillation. The record starts with the auricle in fine fibrillation, a condition produced in this instance through simultaneous stimulation of the right vagus. When vagal stimulation was stopped the auricle gave several large contractions (1, 2, 3) simulating coordinated beats, after which it lapsed into a coarse fibrillation.

To facilitate reference to specific waves, the ventricular systoles as indicated by the R peaks in the electrocardiogram and the corresponding sound vibrations in the myogram are marked alphabetically. Following each R peak in the electrocardiogram a wave corresponding in time and contour to a T wave must be recognized.

During fine fibrillation the auricular myogram clearly shows a series of small mechanical variations irregular in period and amplitude. The electrocardiogram also shows small rhythmic variations. They are only occasionally related to the mechanical shortening. Thus between Systoles A and B only eleven distinct mechanical variations occur while in the electrocardiogram at least sixteen waves are present. Of the mechanical variations two (x′) are definitely preceded by electrical waves(x); but two other distinct mechanical waves (w, y) are entirely without corresponding electrical waves.

Toward the end of the record where coarse fibrillation had taken place the auricular myogram shows a series of larger but still irregular mechanical contractions. If we eliminate the variations of the electrocardiogram which probably represent T waves, there remain very few waves that correspond to the mechanical contractions.

Separating the interval of coarse and fine fibrillation is an interval where several coarse beats occur (1, 2, 3) which somewhat resemble coordinated beats. A comparison of the electrical variations for these beats indicates that Wave 1 is possibly preceded by a corresponding electrical variation; Wave 2 is certainly preceded by no such wave, while it is impossible to determine whether the electrical variation accompanying Wave 3 represents a T wave or an auricular wave.

A study of the curves makes it evident (1) that while large electrical variations may be present during coarse fibrillation they are by no means a constant accompaniment or diagnostic of this condition, (2) that distinct oscillations in the electrocardiogram more frequently accompany fine fibrillation, and (3) that the lack of relation between mechanical and electrical variations during ventricular diastole are characteristic of auricular fibrillation.

Fig. 3, taken on June 25, 1915, shows in order, the external jugular pulse, the auricular myogram, and the intra-auricular pressure curve. The auricles were in a state of coarse fibrillation at the beginning of the record. Then the left vagus was stimulated, resulting in a state of finer fibrillation together with a slower ventricular rhythm.

The curves are of interest as showing first, the influence of mechanical auricular movements on the jugular and venous pressure during long diastoles. The case duplicates the clinical cases slowed by digitalis medication. The beginning of each ventricular systole is indicated by a series of large vibrations in each record (A). The end of systole can be determined approximately by the fall of intra-auricular pressure (B). During the first long diastolic period (1–2) the auricular myogram contains only very small variations, and except for an expected gradual rise of pressure the intra-auricular curve shows no oscillations. Each ventricular systole, however, exerts a definite traction on the auricle consisting of a primary vibration (a–b–c–d) and a slower secondary movement (d–e–f). It will be

noticed that waves corresponding to these tractions are communicated to the jugular vein, and are shown in the upper record. This indicates that ventricular systole, which in the normal heart exerts some traction upon the auricle, pulls even more vigorously upon the distended auricle and veins during fibrillation. Early in diastole a decremental series of oscillations occurs in the auricular myogram (g–h–i–j–k). As similar waves occur in long normal cycles, they can only be interpreted as due to position changes of the relaxing ventricle in both cases. It is evident that these waves are sometimes accompanied by waves in the venous pulse.

Experiments of this nature show that: (1) When diastole is long few wavelets in the myogram or in the jugular can be ascribed to the fibrillating auricle itself. (2) Both ventricular systole and diastole by active traction and recession are capable of producing a passive shortening and lengthening of the auricular fibers, and these may produce similar effects on the veins. This traction communicated to the veins is at least one cause of the large diastolic waves occurring during fibrillation.

Fig. 4 is another record taken from the same animal. It shows a state of coarse fibrillation modified by the action of pituitrin, which moderately slowed the ventricle.

The jugular tracing shows a series of rhythmical waves averaging 300 per minute. From such a record the erroneous impression could easily be derived that the auricles were contracting at this rate. A comparison with other records shows that the auricular mechanical contractions were entirely irregular and that the jugular waves were the result of a fortunate interference between the effects of auricular beats and ventricular traction. Upon the waves are superimposed smaller vibrations which upon careful study can be assigned only to heart sound vibrations. Such tracings show that large coarse rhythmical waves in the jugular must be guardedly accepted as evidence of regular auricular activity.

Fig. 5 shows four segments of a tracing taken on March 8, 1916. The upper curve is a record of the movements of the superior vena cava; the second curve is an auricular myogram; while the lower record is an electrogram obtained by leading off from the two auricular points to which the myogram was attached.

Segment A shows a normal cycle obtained during stimulation of the right vagus. The auricular myogram has the contour described by one of us (Wiggers[4]) as characteristic for a coordinated beat. Ventricular systole, the duration of which is indicated by the two sound vibrations (1S–2S) in the superior vena cava record, causes a jog (a) and several secondary waves (a–b–c–d) on the relaxation curve of the myogram. During early ventricular diastole another wave (d–e–f) is added. These tugs are transmitted to the wall of the superior vena cava as shown in the upper record. Slightly preceding the auricular myogram, the auricular electrogram shows a diphasic variation (1–3) evidently of auricular origin. This is followed by an R peak derived from ventricular activity. As no electrical variations accompany the secondary waves of the myogram (a–b–c–d–e–f) they cannot be assigned to an active contraction, but must be due to passive traction.

Segment B was taken during a state of auricular tachyrhythmia (flutter) induced by previous tetanic stimulation of near points on the right auricle. Each ventricular systole is indicated by a small positive peak (R) in the electrocardiogram and by a series of sound wavelets (S) in the superior vena cava record. In contradistinction to the secondary wavelets following a normal vagus beat, for example, each mechanical variation in the myogram is accompanied first by a small positive, then by a large negative variation in the electrogram. The incidence of irregularly recurring ventricular systoles results in irregular traction upon the auricle and hence the auricular myogram waves during flutter appear of irregular amplitude.

Segment C shows the transition of an existing auricular flutter to a coarse fibrillation upon stimulating the right vagus. The myogram as well as the superior vena cava record still shows a series of small waves which could not have resulted from a ventricular traction, for (1) complete stoppage of the ventricles had occurred, and (2) the electrogram shows a rapid series of irregular variations.

Segment D shows a portion of the curve after vagus stimulation had ceased. The auricle lapsed into a state of coarser fibrillation and the ventricle resumed its beat. Owing to the large auricular waves present in the electrogram derived directly from the auricle, the ventricular systoles are difficult to differentiate in the electrogram,

but are clearly marked by sounds (S) in the superior vena cava record. While it is probable that some of the mechanical variations of the myogram are due to the traction of these ventricular systoles, the presence of the numerous electrical variations of undoubted auricular origin shows that many of the mechanical variations must have been due to the coarse fibrillation of the auricle itself.

A study of these records shows that: (1) Ventricular position changes may cause a series of passive mechanical changes in the auricle (unaccompanied by an action current) which may be the source of some diastolic variation in the venous system. (2) Since the waves during auricular flutter and coarse fibrillation are accompanied by electrical variations and since these waves may occur when ventricular activity is entirely in abeyance, it follows that not all the mechanical variations in the myogram and superior vena cava can be accounted for by ventricular traction. On the contrary, it must be assumed that during coarse fibrillation the impulses travel irregularly over the auricle and excite the various sections of auricular musculature to irregular active contractions, and that these contractions by drawing upon the veins cause large oscillations to appear in the venous pulse.

SUMMARY.

With the clinical recognition that different degrees of fibrillation occur and that these in turn are closely related to a coordinated type of auricular tachyrhythmia (flutter); further, that one type may lapse into another or into a perfectly normal rhythm, the conviction has grown that finer and coarser types of auricular movement may be recognized by the amplitude of the diastolic waves of the electrocardiogram and venous pulse. The present investigation into the cause of these waves has shown that this is not possible. There is no theoretical or experimental reason for the assumption that any fixed relation exists between the amplitude of the electrical variations of the electrocardiogram, which are the resultant of variations accompanying the irregularly spreading excitation wave, and the degree of mass contraction following. The large recurrent waves of the venous pulse, which may with more reason be regarded as related to the size of the auricular mechanical contractions on theoretical

grounds, are also shown to be without differential value. The reasons for this may be briefly summarized.

In the first place, the presence of diastolic waves is contingent upon a slow heart rate and long ventricular diastoles. With rapid heart rate their occurrence is prevented by the closely placed systolic variations. It is therefore conceivable that both coarse and fine fibrillation as well as flutter will be without diastolic waves as long as the heart is rapid.

Even in the cases with long beats present their significance must remain doubtful. It is true that in the experiments the waves accompanying coarse fibrillation are as a rule somewhat larger than those occurring during fine fibrillation; waves of considerable size may, however, be present in fine fibrillation. Hence, as long as no calibrated method of recording is possible, it is difficult to draw any inference. It is possible from the same patient to record with the same apparatus diastolic waves of varying amplitude by merely changing the pressure of the receiving tambour.

The chief reason that the amplitude of these waves cannot be regarded as of differential value is found in their origin. Fine fibrillating movements of the auricle do not in themselves produce waves in the jugular. They produce neither pressure variations in the auricle, nor exert any traction upon the veins. The only factor capable of producing jugular waves during fine fibrillation seems to be the traction exerted by the position changes of the ventricle on the auricle and large veins. This may, in a measure, explain why the diastolic waves recorded from the apex and second left interspace of patients often closely correspond with those simultaneously recorded from the jugular. The term "fibrillary waves" commonly applied to the smaller of these variations is evidently poorly chosen when their etiology is considered. The coarser contractions of the auricle during coarse fibrillation also produce no pressure changes within the auricle. They are vigorous enough at times, however, to exert a traction upon the venous walls. Hence, the waves during coarse fibrillation may be regarded as partly of ventricular and partly of auricular origin, or, as is frequently the case, as due to an interference of the two tractions. It is owing to their dual origin that they are more numerous and distinct when recorded from the same animal without changing the position or pressure of the receiving apparatus.

EXPLANATION OF PLATES.

PLATE 5.

FIG. 1. Intra-auricular pressure, jugular pulse, and auricular myogram, showing transition from fine to coarse fibrillation and from this to coordinated contractions. Diastolic waves of venous pulse (a) occur during fine as well as coarse fibrillation. Shortening of the auricle causes downward movement in this and all other myogram curves. Time 0.2 second.

FIG. 2. Auricular myogram (downstroke in systole) and electrocardiogram, Lead II, showing that diastolic waves of the electrocardiogram do not correspond to mechanical changes of the auricle and that waves are more numerous during fine fibrillation.

PLATE 6.

FIG. 3. Jugular pulse, auricular myogram, and intra-auricular pressure showing the traction effect of the ventricle on the auricle and venous pulse (p. 27).

FIG. 4. The same as Fig. 3 (p. 28).

PLATE 7.

FIG. 5. Four segments of a record showing the movements of the superior vena cava (upper curve), a right auricular myogram (middle curve), and an electrogram of the right auricle (lower curve). Time 0.05 second.

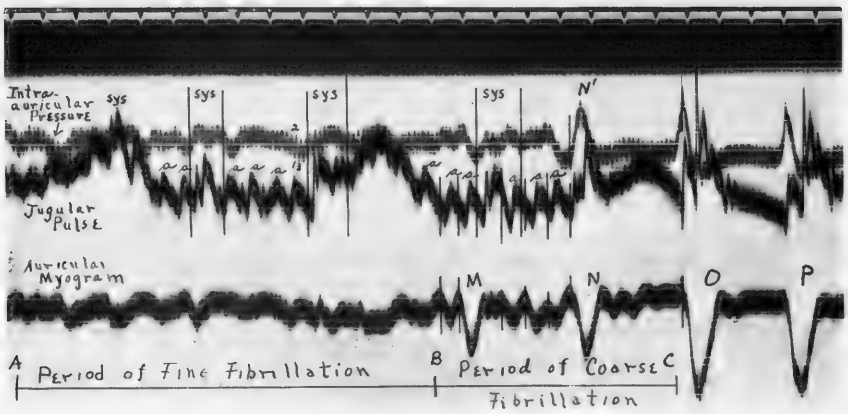

FIG. 1.

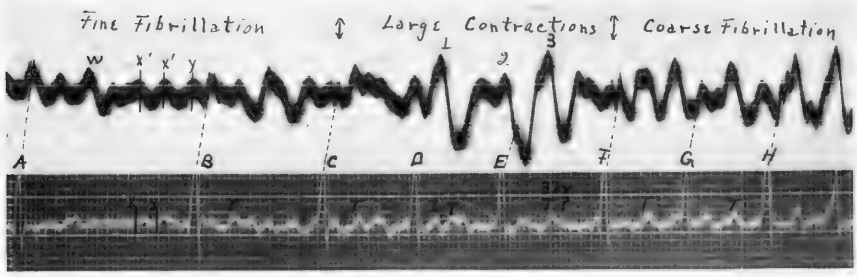

FIG. 2.

(Wiggers and Niles: Diastolic Waves of the Venous Pulse.)

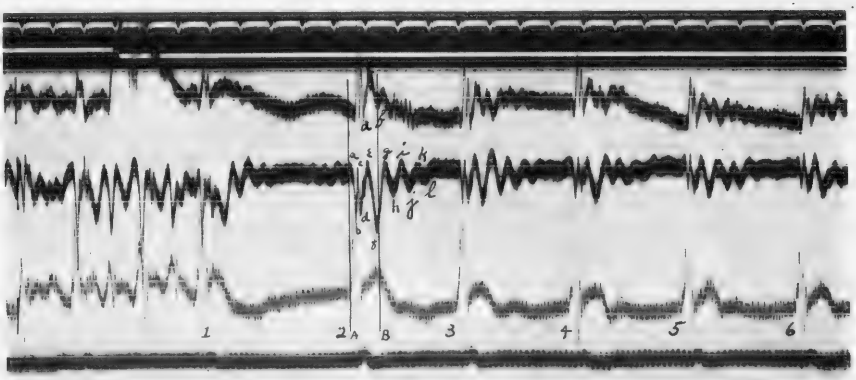

FIG. 3.

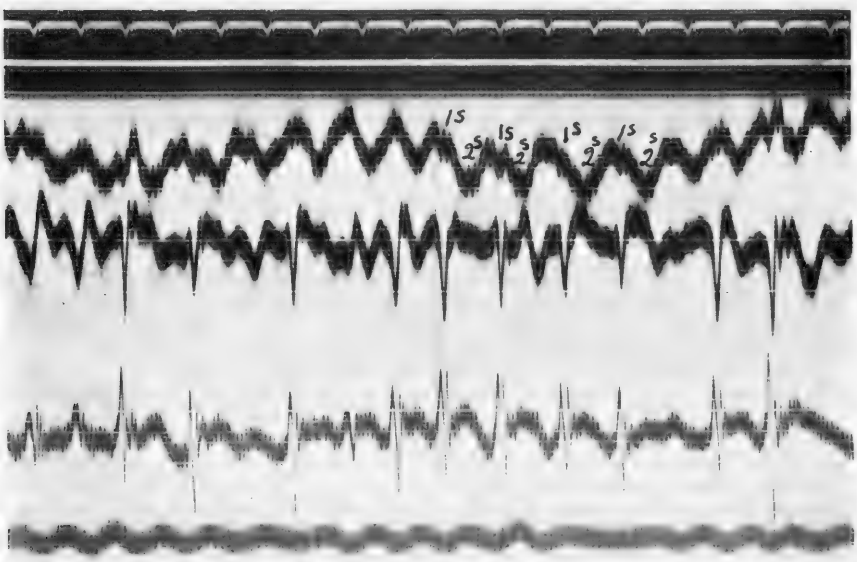

FIG. 4.

(Wiggers and Niles: Diastolic Waves of the Venous Pulse.)

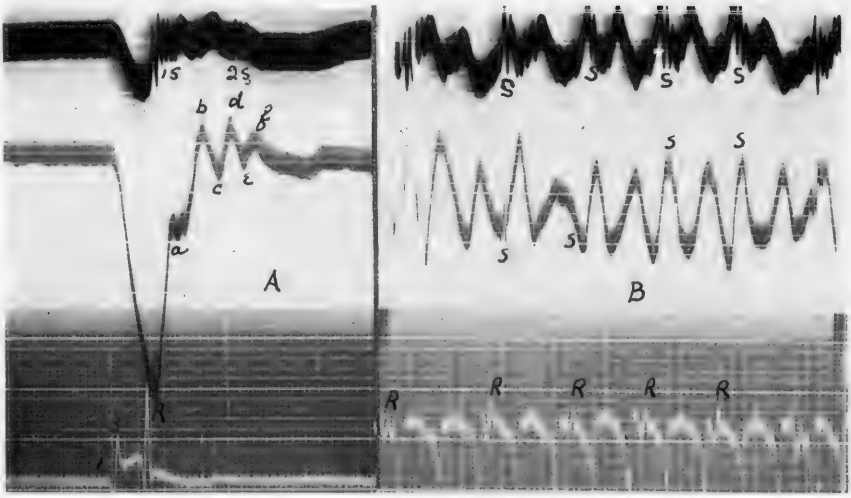

FIG. 5 a.

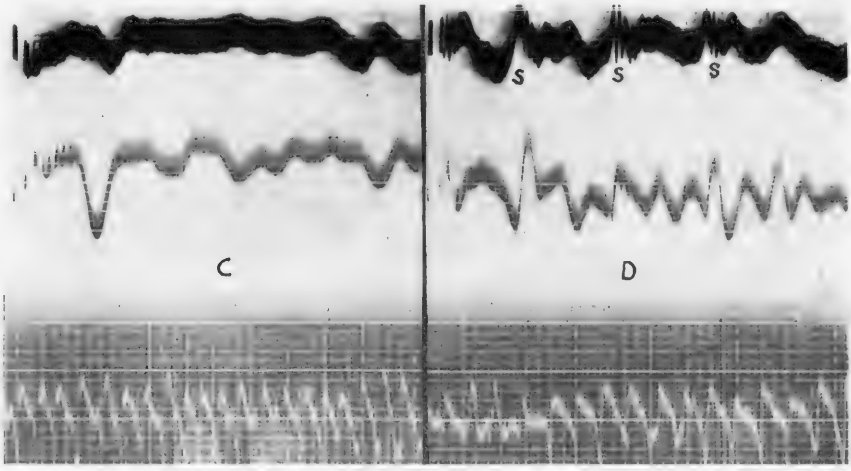

FIG. 5 b.

(Wiggers and Niles: Diastolic Waves of the Venous Pulse.)

SPIROCHÆTA MORSUS MURIS, N.SP., THE CAUSE OF RAT-BITE FEVER.

SECOND PAPER.

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PLATES 8 TO 10.

(Received for publication, August 2, 1916.)

We have already reported the finding of a new spirochete,[1] which we believe to be the cause of rat-bite fever and which was present in the blood, skin, and lymph glands of two out of four patients suffering from the disease. Five more cases, in each of which we have been able to find a spirochete, have come under our observation and in two of the patients we found the spirochete in the circulating blood derived from a vein or the punctured skin.

Description of the Spirochete.

Morphology.—The spirochete consists of a body and flagella. In stained film preparations of the blood of a patient or inoculated animal, the body is spiral and comparatively short and thick. The size varies from 1.5 to 2 μ to 5 to 6 μ; when the flagella at both ends are included, the length is 6 to 10 μ. Smaller bodies are found in the blood in greater numbers than large ones, while in lymph nodes slightly larger ones occur. In cultures still larger ones measuring up to 12 to 19 μ, have sometimes been observed; but minute ones of 1.5 to 2 μ also appear.

In our previous report we stated that the organism in the patient's lymph nodes was somewhat larger than *Spirochæta pallida*, but

[1] Futaki, K., Takaki, I., Taniguchi, T., and Osumi, S., *J. Exp. Med.*, 1916, xxiii, 249.

smaller than *Spirochætæ duttoni* and *obermeieri*. It is thicker also than *Spirochæta pallida* and presents usually one curve per micron, the curves being regular and sharp. The number of curves ranges from one and a half to six; occasionally ten to nineteen curves appear. Other exceptions are as follows: in cultures the angle of the curves is obtuse; some spirochetes show one curve in 2 μ. In rare instances, especially in cultures in which degeneration quickly occurs, the curves become irregular. The characteristics are the same whether living preparations or stained films are studied. An undulating membrane has not been found. The protoplasm stains equally well, regardless of the stain used. The ends of the body gradually become pointed and pass into the slender flagella. In most cases flagella project from both ends; sometimes from one only. Rarely two flagella occur at one end. The flagella have approximately the same thickness and measure 2 to 3 μ.

Staining.—The spirochetes stain readily. Giemsa's stain, Löffler's methylene blue, Ziehl's solution, and aqueous gentian violet all stain the organisms readily. They are, however, Gram-negative. Silver impregnation succeeds easily. The flagella may or may not become stained with Giemsa's stain or silver solution. India ink, used according to Burri, is very effective in revealing the flagella. With Giemsa's stain the bodies take a deep violet-red coloration. Levaditi's method of silver impregnation is most suitable for demonstrating the presence of the spirochete in the tissues. By this method the bodies appear thick and the curves sharper, giving the organism a spindle-like appearance. In this case, the spirochetes can be distinguished only by means of the flagella which assume a yellowish brown color. In some culture organisms one or more chromatin bodies have been detected by means of Giemsa's stain.

Locomotion.—Usually it is difficult to find the spirochetes in the blood or in the lymph of patients even by means of dark-field illumination, as the number is small. Recently, however, we observed in one case out of five, spirochetes in blood from the punctured skin. We have, on the other hand, experienced difficulty in finding the organisms in thick blood preparations made according to Koch's method. In the inoculated mouse it is not infrequent to find from one to five spirochetes in a single field of blood or lymph. Hence the number of

spirochetes is far less than in other spirochetal infections communicated to animals.

The light refraction is weak and the motion is like that of a vibrio. The organism moves rapidly across the field of vision; but the cultivated spirochetes with long bodies are, on the contrary, inactive, making only wriggling movements.

Movements may be clearly discerned in specimens studied according to Shimamine's[2] method. The agar medium, after having been dissolved and cooled to 45°C., is mixed with fresh blood containing spirochetes. This mixture is placed under a cover-glass and the spirochetes can be seen fixed in the agar; capillary preparations are then made. The movements are spasmodic, usually spiral, and freely pass backwards and forwards. Comparatively long organisms undergo flexion and rotation. Meanwhile, whipping, winding, stretching, and darting motions of the flagella can be seen. When the bodies are very small it is often impossible to distinguish the various movements.

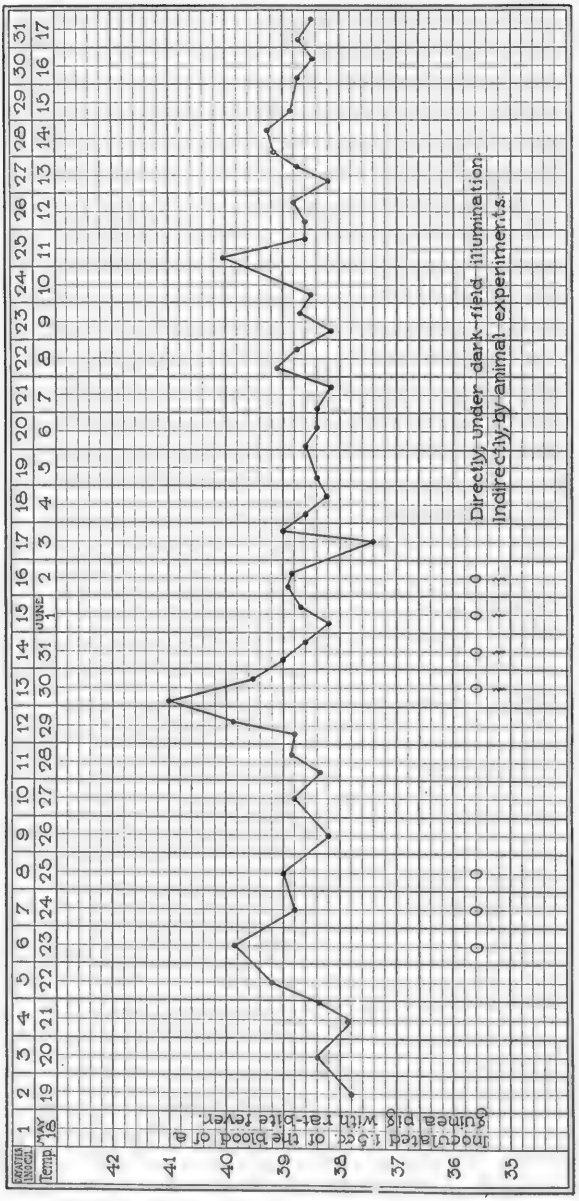
The spirochete examined microscopically under a strong light becomes quiescent comparatively soon. In animal blood at a temperature of 22–37°C., it ceases to move in about 24 to 48 hours. In this condition it is not recognizable under the dark-field microscope.

Mice, house rats, white rats, guinea pigs, and monkeys are suitable for the experiments, but mice are the best, especially when human material is to be inoculated directly into animals. White rats are the next best. Guinea pigs and monkeys frequently yield no result.

Spirochetes which have passed through the blood of mice or rats several times are highly virulent for other animals. This virulence depends, of course, largely upon the number of spirochetes inoculated.

After the inoculation of a mouse with human material, 7 days at the earliest, and several weeks or a month at the latest elapse before the organisms can be discovered in its blood. But if a mouse is in-

[2] Shimamine's medium is prepared as follows: 0.5 to 0.75 gm. of sodium nucleate and 100 cc. of horse serum are shaken until the former is completely dissolved, after which carbon dioxide is passed through the solution for 3 or 4 minutes, until the serum becomes transparent. The liquid is heated on 3 successive days, for about an hour at 60°C. On the 4th day it is heated at 65°C. for about 30 minutes, when it separates into a fluid and a coagulated portion.


Temp.
MAY
JUNE
Inoculated 1.5 cc. of the blood of a guinea pig with rat-bite fever.
Directly under dark-field illumination.
Indirectly by animal experiments.

TEXT-FIG. 1. *Pithecus fuscatus* (Blyth) inoculated with the blood of a guinea pig suffering from rat-bite fever.

oculated from an infected mouse, the spirochetes usually begin to appear in 4 days. Mice usually survive the experiment and retain the spirochetes for several months after the inoculation. Although the other animals generally survive the disease, death occurs now and then from various causes. Swelling of the spleen, hyperemia, congestion and swelling of the liver, or hemorrhage of the kidneys and lungs are often observed. Many of the organisms are also found in the liver or kidneys of animals as well as in their blood. The type of fever produced in the monkey, by the inoculation of spirochetes of rat-bite fever, is not typical of the fever in man. However, the type of fever in the monkey caused by inoculation with the spirochetes of relapsing fever is not typical of relapsing fever in man.

Propagation of the Spirochetes in the Human Body.

As cases of rat-bite fever are rare—only eight have come under our observation—and as we have not yet examined a fatal case,[3] our investigation is still far from complete. In the early stages the spirochetes are found in the local eruption of the skin and in the enlarged lymph nodes, whence they seem gradually to invade the blood.

In relapsing fever the organisms are numerous during the febrile, but decrease greatly in number or disappear altogether during the afebrile periods. When animals are inoculated with blood from a patient suffering from rat-bite fever during the afebrile period or when there is light fever, the spirochetes are present. However, it is always difficult to find them as they are few in number.

EXPERIMENTAL.

A. The striking clinical effect of salvarsan upon rat-bite fever has already been reported by Taniguchi and Hata,[4] whose experiments we have confirmed. We divided into three groups a number of mice in the blood of which the spirochetes had been demonstrated. The first group was kept as a control, and each animal in the other two

[3] A postmortem has recently been reported from the Kyushu Imperial University by Drs. Kaneko and Okuda. They claim to have found the same organism as ours in the intestines, and especially in the kidneys.

[4] Hata, S., *Münch. med. Woch.*, 1912, lix, 854.

groups was injected with $\frac{1}{600}$ to $\frac{1}{800}$ gm. of salvarsan for every 20 gm. of body weight (Table I).

TABLE I.

Experiments with Salvarsan.

Hypodermic Injection of 0.2 Cc. of Blood Containing Spirochæta morsus muris.

Mouse No. Body weight.	1	2 Control.	3	4 12 gm.	5 13 gm.	6 12 gm.	7 12 gm.	8 13 gm.	9 14 gm.
Dose per 20 gm. of body weight.				$\frac{1}{600}$ gm.			$\frac{1}{800}$ gm.		
No. of spirochetes.									
1st day (Mar. 2).	−	+	+	+	+	+	+	+	+
2nd "	+	+	+	−	−	−	−	−	−
3rd "	+	+	+	−	−	−	−	−	−
4th "	+	+	+	−	−	−	−	−	−
5th "	+	+	+	−	−	−	−	−	−
6th "	+	+	+	−	−	−	−	−	−
7th "	+	+	+	−	−	−	−	−	−
8th "	+	+	+	−	−	−	−	−	−
9th "	+	+	+	−	−	−	−	−	−
10th "	+	+	+	−	−	−	−	−	−
11th "	+	+	+	−	−	−	−	−	−
12th "	+	+	+	−	−	−	−	−	−
13th "	+	+	+	−	−	−	−	−	−
14th "	+	+	+	++	−	−	−	−	+
15th "	+	+	+	+	−	−	−	+	+
16th "	+	+	+	+	+	−	−	+	+

On the following day, though the spirochetes were found as before in the blood of the control animals, none were discovered in the other groups. This result continued until the 14th day, when about one-third of the animals showed a relapse; by the 17th day almost two-thirds had relapsed, and after 2 months 83 per cent showed spirochetes. A similar phenomenon has been witnessed in human beings.

Occurrence of the Spirochete in Nature.

We examined the blood of forty-three rats under dark-field illumination, and in one only, namely, a *Mus rattus alexandrinus* (roof rat) did we detect spirochetes. With the blood of this rat a white mouse was inoculated successfully. In respect to morphology, staining,

locomotion, and infectivity the spirochetes present in the roof rat are identical with those obtained from human cases of rat-bite fever. Yet we have not discovered the organism in the mouths of healthy house rats or in the saliva of house rats and other animals in whose blood the spirochete exists.[5] The spirochete found in a *Mus decumanus* by Carter[6] in 1887 and again by Mezinescu[7] in 1909 may be the same as ours. Moreover, Borrel,[8] Calkins,[9] and others have described spirochetes in cancerous and healthy mice. In one instance the description fitted the organism we are considering, but it has not been detected up to the present in healthy mice or guinea pigs in Japan.

B. We discovered our organism in November, 1915. Soon after, Ishiwara and his associates succeeded in cultivating outside the body a spirochete which was transmitted by the bite of a house rat to a guinea pig. In this case the spirochete-containing blood of the guinea pig was used. We have made similar observations on a somewhat larger scale. Some fifteen house rats having bitten guinea pigs, we examined the blood of the latter and found in one instance spirochetes which were identical with those present in rat-bite fever in man.

C. From the fact that patients suffering from rat-bite fever have invariably been bitten by rats and also that our spirochetes are found both in house rats and in human patients with rat-bite fever, it may be inferred that the spirochete is transmitted from these animals to the human body, and the experiments described above confirm this view. But as the organisms have not been detected in the saliva but only in the blood of rats, the source of the infectious organism probably is from the blood which, owing to hemorrhage from the gums, enters the saliva of the rat.

D. Various methods have been tested for the cultivation of the

[5] We owe to Drs. Ishiwara, Otawara, and Tamura valuable specimens of spirochetes from two rats—a *Mus decumanus* and a *Mus rattus alexandrinus*—in which they detected the organism.

[6] Carter, V., *Sc. Mem., Med. Officers Army of India*, 1887, iii, 45.

[7] Mezinescu, D., *Compt. rend. Soc. biol.*, 1909, lxvi, 58.

[8] Borrel, A., *Compt. rend. Soc. biol.*, 1905, lviii, 770.

[9] Calkins, G. N., and Clowes, G., *J. Infect. Dis.*, 1905, ii, 555.

spirochete, including especially Noguchi's and Shimamine's[2] employed for the cultivation of *Spirochæta pallida*. We have succeeded in cultivating our organism with the latter method, the first appearance of growth being noted after 14 days at 37°C. The inoculation is made by thrusting into the medium, thus prepared, a capillary tube impregnated with the blood of an animal containing the spirochetes. The tubes are not paraffined, and are left in the thermostat at 37°C. for 2 weeks. The growth is attended neither by liquefaction nor other obvious changes in the medium and no odor is developed. Multiplication is detected first by the dark-field microscope, and secondly by stained preparations. The pathogenicity of the cultures is under investigation.

The cultures show the following characteristics. The maximum number of spirochetes found in a microscopic field of the blood used for inoculation was three to four; of the culture, six to ten. The blood having been placed in the center of the medium, the spirochetes are diffused in the medium to within 1 cm. of the surface. Should the spirochetes not multiply in the culture those inoculated disappear in a week at the longest. Thus far a second culture generation has not been secured.

The size of the culture organisms varies greatly—the short ones measure from 1.5 to 2 μ, the large ones from 12 to 19 μ. The spirals are fewer in number (one curve in 2 μ) than in the blood spirochetes. The total number of curves varies from 1 to 1.5 to 19. The larger individuals in cultures have not been found either in the blood or lymph glands.

The culture spirochetes possess the same staining reaction as those of the blood. All the aniline dyes stain them, and Giemsa' solution gives them a deep violet-red tint. The flagella also stain distinctly. The larger organisms seem to divide transversely; vertical division has not been observed. Between the bodies slender, light colored mid-lines have been seen. In one instance one body was noted to give rise to four bodies. We have occasionally seen organisms with irregular spirals unevenly stained, which might be considered as degenerate forms. Once we observed from one to several deep red refractive chromatin corpuscles scattered among the culture spirals. In one instance only have we seen two flagella at one end of the body.

The culture organisms are motile—movements of the short individuals are brisk and similar to those of vibrios; the larger spirochetes exhibit slow, wriggling or whipping movements. 0.1 per cent sodium taurocholate and saponin dissolves the organism.

As regards classification, we believe that the spirochete belongs to the protozoa and not to the bacteria. We have not yet succeeded in obtaining a second generation of the culture.

Spirochetes have been described as occurring in rats or mice either in the blood or in tumors by many investigators (Carter,[6] Borrel,[8] Calkins[9] Wenyon,[10] Breinl and Kinghorn,[11] MacNeal,[12] Tyzzer,[13] Gaylord,[14] Deetjen,[15] Löwenthal,[16] Mezinescu,[7] Negre, (1910), and Hoffmann (1906). Although only a part of the descriptions suffices for certain identification, we have not been able to identify our organism with any of them. Moreover, in the case of none of the spirochetes described has the question of human infection entered. Hence we have concluded to offer as a designation of the spirochete of rat-bite fever the name *Spirochæta morsus muris*.

Ogata believes that rat-bite fever is caused by an aspergillus; Shikami[17] that it is caused by a telosporidia; Middleton,[18] by a diplococcus; Proescher,[19] by bacteria; and Schottmüller,[20] by a streptothrix. The streptothrix theory of Schottmüller was supported separately by Blake[21] and Tileston, and also by Tunnicliff.

By rat-bite fever we mean a disease having the symptoms described above, but the fever does not necessarily include all diseases related to rat bites. Hence we consider that the cause of rat-bite fever in this sense is the spirochete that we have described. In Schottmüller's two cases in which the victims fell ill without an incubation period, the disease may have been caused by a streptothrix, but it is not what we should call rat-bite fever. Tileston's cases were appar-

[10] Wenyon, C. M., *J. Hyg.*, 1906, vi, 580.
[11] Breinl, A., and Kinghorn, A., *Lancet*, 1906, ii, 651.
[12] MacNeal, W. J., *Proc. Soc. Exp. Biol. and Med.*, 1906–07, iv, 125.
[13] Tyzzer, E. E., *Proc. Soc. Exp. Biol. and Med.*, 1906–07, iv, 85.
[14] Gaylord, *Ann. Rep. Cancer. Lab., New York State Dept. Health*, 1907, viii, 34.
[15] Deetjen, H., *Münch. med. Woch.*, 1908, lv, 1167.
[16] Löwenthal, W., *Berl. klin. Woch.*, 1906, xliii, 283.
[17] Shikami, *Z. med. Ges. Tokyo*, 1909.
[18] Middleton, G. S., *Lancet*, 1910, i, 1618.
[19] Proescher, F., *Berl. klin. Woch.*, 1912, xlix, 841.
[20] Schottmüller, H., *Derm. Woch.*, 1914, lviii, Suppl. 77.
[21] Blake, F. G., *J. Exp. Med.*, 1916, xxiii, 39.

ently the same as ours, but he failed to detect the spirochete. Blake's case differs from our cases of rat-bite fever in that the lymph glands were not swollen, the rash was temporary, and the fever was not paroxysmal; it may properly be regarded as a case of streptothrix sepsis caused by the bite of a rat.

SUMMARY.

1. Since our first report on the discovery of the cause of rat-bite fever, we have been able to prove the existence of the same spirochete in five out of six more cases which have come under our observation.

2. The clinical symptoms of rat-bite fever are inflammation of the bitten parts, paroxysms of fever of the relapsing type, swelling of the lymph glands, and eruption of the skin, all occurring after an incubation period usually of from 10 to 22 days, or longer.

3. Our spirochete is present in the swollen local lesion of the skin and the enlarged lymph glands. But as the spirochetes are so few in number it is exceedingly difficult to discover them directly in material taken from patients. It is therefore better to inoculate the material into a mouse. In some cases the organism is found in the blood of the inoculated animal after a lapse of 5 to 14 days, or at the latest 4 weeks.

4. Generally speaking, the spirochetes present thick and short forms of about 2 to 5 μ and have flagella at both ends. Including the flagella, they measure 6 to 10 μ in length. Some forms in the cultures reach 12 to 19 μ excluding the flagella. The curves are regular, and the majority have one curve in 1 μ. Smaller ones are found in the blood and larger ones in the tissues.

5. The spirochetes stain easily. With Giemsa's stain they take a deep violet-red; they also stain with ordinary aniline dyes. The flagella, too, take Giemsa's stain.

6. The movements of our spirochetes are very rapid, resembling those of a vibrio, and distinguish them from all other kinds of spirochetes. When, however, the movements become a little sluggish, they begin to present movements characteristic of ordinary spirochetes.

7. For experimental purposes, mice, house rats, white rats, and monkeys are the most suitable animals. Monkeys have intermittent fever after infection, and spirochetes can be found in their blood, but

they are not so numerous as in the blood of mice. Mice are the most suitable animals for these experiments, and they appear, as a rule, to escape fatal consequences.

8. The spirochete is markedly affected by salvarsan.

9. The organism is not present in the blood of all rats, and there is no relation between the species of the rat and the ratio of infection. We have never found the spirochete in healthy guinea pigs or mice. By permitting a rat infected with the spirochete to bite a guinea pig, the latter develops the disease.

10. We have succeeded in cultivating the spirochete in Shimamine's medium.

11. Among the spirochetes described in the literature or discovered in the blood of rats and mice, there may be some resembling our spirochete, but none of the descriptions agree with it fully. Hence we have named our organism *Spirochæta morsus muris* and regard it as belonging to the Spironemacea (Gross) of the nature of treponema.

12. The spirochete can be detected in the bodies of patients. In seven cases out of eight, it disappears on recovery, only to reappear during the relapse.

13. The spirochete can be detected in about 3 per cent of house rats. These facts enable us to identify the cause of the disease.

14. There may be other causes than the spirochete for diseases following the bite of a rat. The cause, however, of rat-bite fever in the form most common in Japan is, we believe, the spirochete which we have described.

In conclusion we wish to express our indebtedness to Dr. Aoyama, former Director of the Imperial Institute for Infectious Diseases, and Dr. Hayashi, the present Director; to Dr. Miura who named the spirochete; to Professor Nagayo, Assistant Professor Ishiwara, and Drs. Manabe, Miyagawa, Mitamura, Matsuyama, and Katayama, for valuable suggestions; and to Dr. Oba, and Messrs. Itakura and Isosaki for the help they have given us.

EXPLANATION OF PLATES.

PLATE 8.

FIG. 1. Section of a lymph gland from a patient with rat-bite fever. Impregnated with silver nitrate according to Levaditi's method. × 1,250.

FIG. 2. Section of the lung of a mouse inoculated with venous blood from a patient with rat-bite fever. The length of the body of the spirochete is 2.2 μ; including the flagella it is 6 μ. Silver impregnation. $\times$ 1,500.

FIG. 3. A spirochete from a guinea pig with experimental rat-bite fever. The length of the body is 4μ.; with the flagella, 8.5. Giemsa's stain. $\times$ 1,500.

FIG. 4. Spirochetes from a guinea pig with experimental rat-bite fever. The length of the bodies varies from 2.2 to 4 μ. Giemsa's stain. $\times$ 1,250.

PLATE 9.

FIG. 5. *Spirochæta morsus muris* from a mouse inoculated with the blood of a patient with rat-bite fever. The length of the body is 4 μ; with the flagella it measures 9 μ. $\times$ 3,600.

PLATE 10.

FIG. 6. First column, *Spirochæta morsus muris* from the blood and tissues of human patients and inoculated animals. Second and third column, cultures of *Spirochæta morsus muris*.

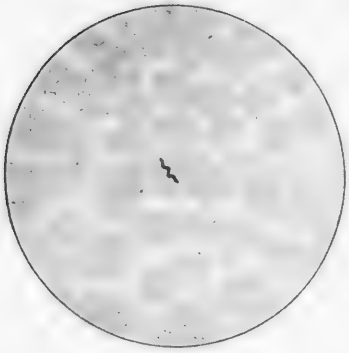


FIG. 1.

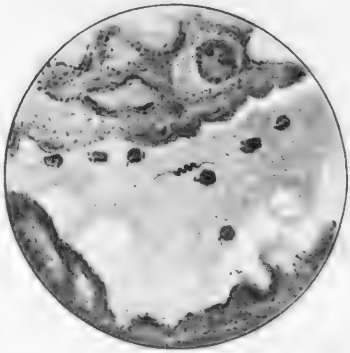


FIG. 2.

FIG. 3.

FIG. 4.

(Futaki, Takaki, Taniguchi, and Osumi: Cause of Rat-Bite Fever.)

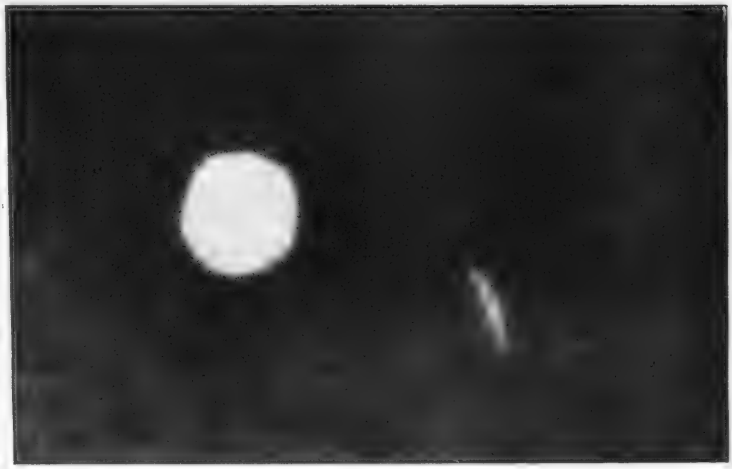


FIG. 5.

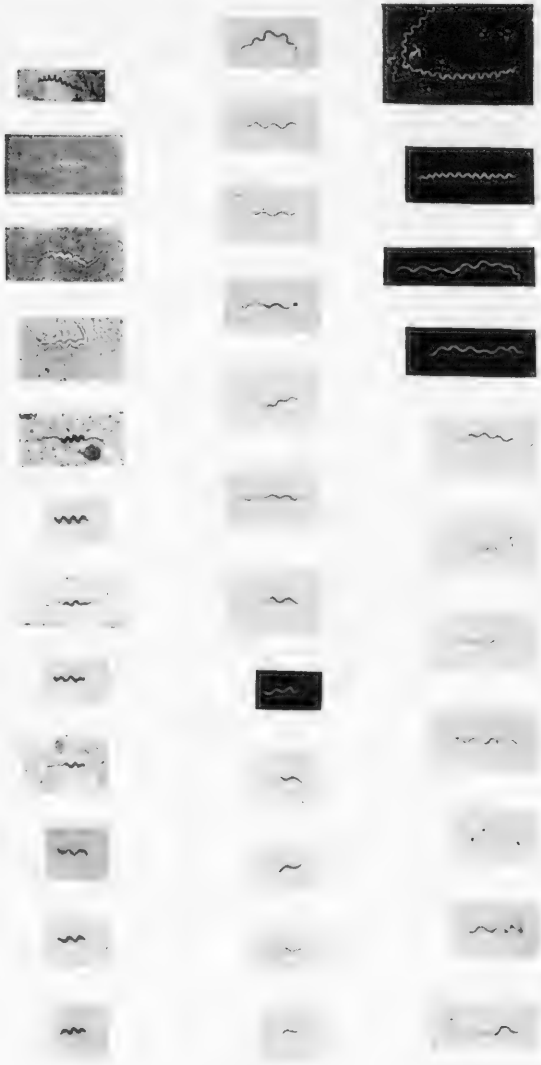


FIG. 6.

(Futaki, Takaki, Taniguchi, and Osumi: Cause of Rat-Bite Fever.)

EXPERIMENTAL RAT-BITE FEVER.

First Report.*

By KIKUTARO ISHIWARA, M.D., TOYOITSIRO OHTAWARA, M.D., and KOTARO TAMURA.

(*From the Imperial Institute for Infectious Diseases, Tokyo, Japan.*)

Plate 11.

(Received for publication, August 7, 1916.)

INTRODUCTION.

At least eighty-one cases of human rat-bite fever have, according to Blake,[1] been reported by European, British, American, and Japanese investigators. Only two of the reports, however, those of Miura and Toriyama[2] and of Blake, include the pathological anatomy of the disease. Ogata[3] attributes rat-bite fever to an aspergillus, Shikami[4] attributes it to a telosporidia, Middleton[5] to a diplococcus, Proescher[6] to a bacillus, and Schottmüller[7] and Blake to a streptothrix.

Ogata inoculated two guinea pigs with a freshly excised piece of a swollen lymph gland taken from a patient with rat-bite fever; both animals died about 3 weeks later. The swollen glands of the animals were inoculated into other guinea pigs which also died about 3 weeks after the inoculation. In 1909 Ogata caused a *Mus decumanus* to bite two guinea pigs in the leg; the guinea pigs developed fever and swelling and congestion of the bitten parts and finally succumbed. Autopsy of all these animals showed swelling and congestion of the lymph glands and swelling of the kidneys to be the chief lesions, although pneumonia was present in some instances. From these experiments Ogata concluded that rat-bite fever could be produced experimentally in animals by the bite of rats.

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* This paper was presented at the meeting of the Japanese Hygienic and Bacteriological Society on March 3, 1916.

[1] Blake, F. G., *J. Exp. Med.*, 1916, xxiii, 39.

[2] Miura, M., and Toriyama, N., *Z. med. Ges. Tokyo*, 1897, xi, 1059.

[3] Ogata, *Mitt. med. Fakult. Univ. Tokyo*, 1911, ix, 1913; 1913–14, xi, 179.

[4] Shikami, *Z. med. Ges. Tokyo*, 1909.

[5] Middleton, G. S., *Lancet*, 1910, i, 1618.

[6] Proescher, F., *Berl. klin. Woch.*, 1912, xlix, 841.

[7] Schottmüller, H., *Derm. Woch.*, 1914, lviii, Suppl. 77.

One of us had the opportunity of assisting at these experiments, and noted pathological changes in the adrenals of the animals, but Ogata made no mention of them in his report.

In March, 1915, one of us undertook the serological study of guinea pigs bitten by rats. In November of the same year Futaki and his collaborators examined under dark-field illumination and by Burri's method the tissue fluid of excised skin and that of punctured lymph glands of two patients suffering from rat-bite fever. They also examined the excised lymph gland of one of the patients, stained by Levaditi's method, and found that it contained a number of spirochetes. About the same time we examined the excised viscera of guinea pigs which we had kept for study from the spring of that year and found a spirochete in sections of the adrenals of several animals, stained according to Levaditi's method. Futaki[8] and his coworkers announced the results of their investigation at a meeting of the Tokyo Medical Society on November 20, 1915, and we reported our findings at the same meeting. We promptly found the same spirochetes in living animals which were under observation and described them in a second report made before the Tokyo Medical Society on February 5, 1916.

Ogata was the first to transmit rat-bite fever to guinea pigs by causing rats to bite them, and we have confirmed his experiments.

EXPERIMENTAL.

We caught many rats by means of wire nets and kept them under observation in the laboratory. The rats were divisible into two classes, those that would and those that would not bite when irritated from time to time by means of a small metal rod. Rats which did not bite in one test would, as a rule, not bite in another. Moreover, only a part of the rats were capable of conferring the rat-bite infection, because a rat which did not confer the disease to one animal by biting did not confer it to another; conversely a rat which conveyed the disease by biting one animal would also convey it by biting another. This led us to conclude that only certain rats tend to bite and of those only a part convey the infection of rat-bite fever.

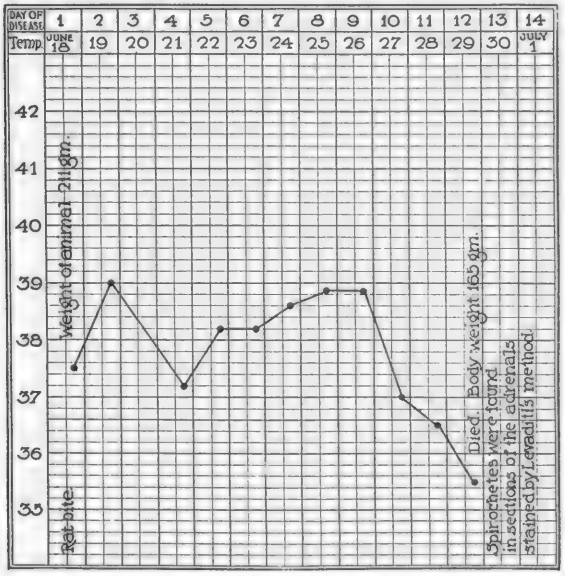
The test animals were guinea pigs the legs of which were exposed. About 80 rats were employed, one-half of which were made to bite, and of this number only about ten individuals, all *Mus decumanus*, caused experimental rat-bite fever. Thus far we have not discovered the exact conditions under which the disease is transmitted, and we have not detected the specific spirochete in the mouths

[8] Futaki, Takaki, Taniguchi, and Osumi, *Z. med. Ges. Tokyo*, 1915.

of rats. In one rat, however, we detected a few spirochetes of the same form in the blood.

Clinical Course.

The symptoms and course of the disease in the guinea pig were as follows: The bitten parts became swollen within 2 or 3 days, and in severe cases the affected legs reached three times the natural size. The bite healed promptly, but the swelling continued, sometimes de-

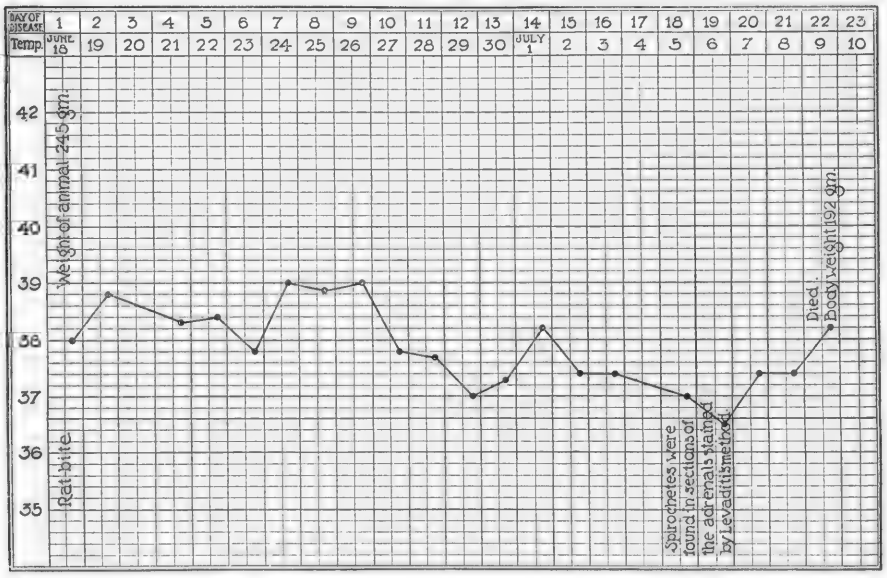

DAY OF DISEASE
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Temp.
JUNE 18 19 20 21 22 23 24 25 26 27 28 29 30 JULY 1
42
41
40
39
38
37
36
35
Weight of animal 211 gm.
Rat bite
Died. Body weight 165 gm.
Spirochetes were found in sections of the adrenals stained by Levaditi's method

TEXT-FIG. 1. Temperature chart of Guinea Pig 1 (original generation).

creasing, until the death of the animal. The skin of the leg became cyanosed and the subcutaneous lymph glands became palpable. Within 24 hours after the bite a rise in temperature occurred, but this was soon followed by a fall to normal. A second rise of temperature took place 6 to 7 to 10 days later, only to fall again soon. This rise and fall might be repeated, but in this respect the experimental condition does not run so regular a course as human rat-bite fever.

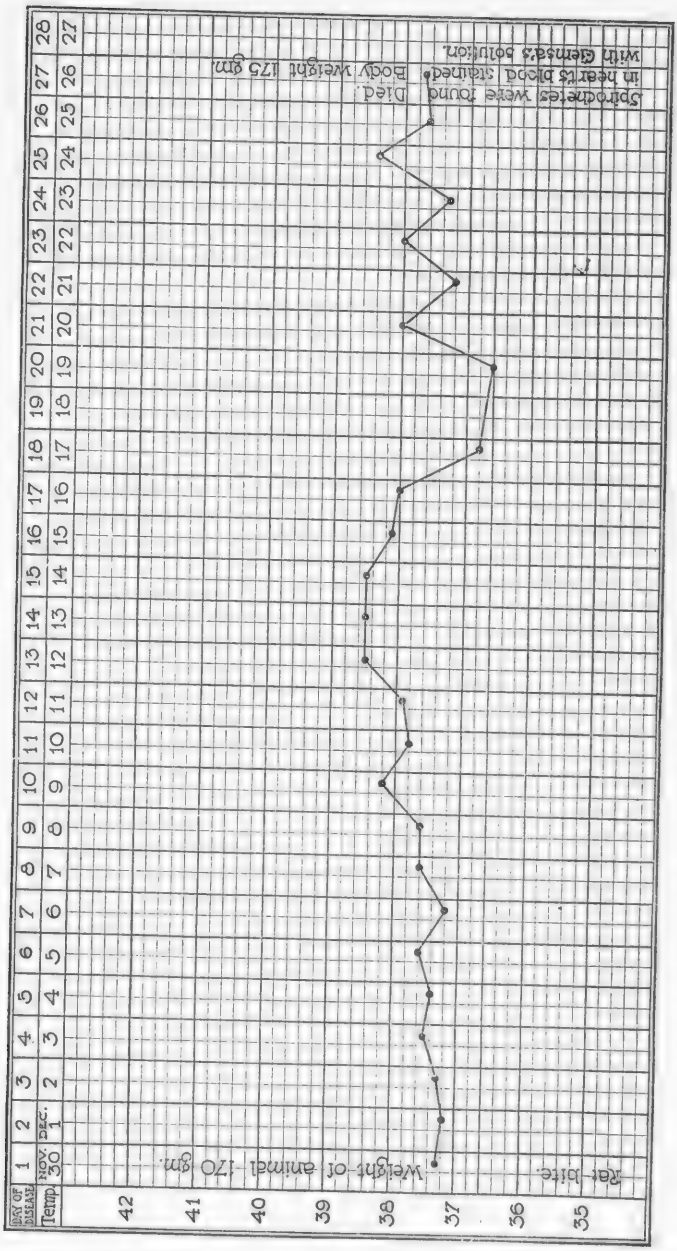
Weight was gradually lost, but an erythema did not appear. The guinea pigs which showed this condition invariably died. The course of the fever was not uniform, but tended to extend over 3 weeks. The longest course was something over 5 weeks, the shortest less than 2 weeks. Text-figs. 1, 2, and 3 show the progress of events in three instances.

Guinea Pig 1 (Text-fig. 1) was bitten by *Mus decumanus* 4 on June 18, 1915. On the 2nd day the bitten parts became swollen and on the 4th day the enlarged inguinal glands on the affected side were palpable.


DAY OF DISEASE
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Temp.
JUNE 18 19 20 21 22 23 24 25 26 27 28 29 30 JULY 1 2 3 4 5 6 7 8 9 10
42
41
40
39
38
37
36
35
Weight of animal 245 grm.
Rat-bite
Spirochetes were found in sections of the adrenals stained by Levaditi's method.
Died. Body-weight 192 grm.

TEXT-FIG. 2. Temperature chart of Guinea Pig. 2 (original generation).

The anatomical appearances are described below. Two fresh guinea pigs were inoculated with emulsions made from the lymph glands and two with emulsions made from brain tissues of this animal. Spirochetes were found in sections of the adrenals stained according to Levaditi's method. Aerobic cultures made after the animal's death from the intraperitoneal exudate, heart's blood, fluids of brain tissues and excised tissues taken from the section through the bitten parts were negative, as were also the stained cover-glass preparations.


Rat bite
Weight of animal 170 gm.
Died.
Body weight 175 gm
Spirochætes were found in heart's blood stained with Giemsa's solution.

TEXT-FIG. 3. Temperature chart of Guinea Pig 3 (original generation).

Before inoculation we always examine the animals' blood microscopically, after treating it with acetic acid.

Guinea Pig 2 (Text-fig. 2) was bitten by the same rat on the same day as No. 1. On the 2nd day the bitten parts became swollen; the swelling decreased soon after, but it increased again on the 9th day. Enlargement of the inguinal glands could be detected on palpation. On the 14th day the swelling of the bitten parts again decreased. The anatomical appearances were identical to those described below. Aerobic cultures made from the excised tissues of the bitten parts, the intraperitoneal fluid, heart's blood, and brain tissue fluid were negative. Stained cover-glass preparations were also negative. Two fresh guinea pigs were infected with emulsions made from lymph glands and brain tissues. Excised tissues of the adrenals stained according to Levaditi's method were positive for spirochetes.

Guinea Pig 3 (Text-fig. 3) was bitten on November 30, 1915, by another *Mus decumanus*. The next day the leg was swollen to three times the normal size and developed a dark cyanotic color. From the 10th day on the fever progressed as shown in Text-fig. 3. The animal died on the 28th day after being bitten.

Autopsy.—Weight of animal not decreased. The leg remained swollen. Swelling and congestion of the subcutaneous and internal lymph nodes were also present. Both adrenals were markedly swollen and congested. The kidneys of both sides showed congestion of the cortex. Aerobic cultures from the peritoneal fluid and heart's blood were sterile. Further inoculation into guinea pigs with the heart's blood and emulsion of the mesenteric lymph nodes gave positive results. Spirochetes were found in the heart's blood stained by Giemsa's solution.

Anatomical Changes.

The subcutaneous lymph glands and the inguinal and axillary glands were swollen and congested. The periglandular tissues and the mesenteric and retroperitoneal glands were also congested. The liver and lungs were congested, the latter often showing pneumonic foci. The spleen showed no apparent macroscopic change. The kidneys were more or less swollen, hemorrhage being often noted under the capsule. The sectioned surface showed edema, and small hemorrhagic spots were observed in the cortical parts. The adrenals were congested and showed small hemorrhagic spots under the capsules. In the peritoneal cavity a hemorrhagic exudate was sometimes found. The bladder and gall-bladder were usually full. The pia mater was congested. The important points to be noted among the postmortem appearances are: swelling of the bitten parts, swelling and congestion of the lymph gland system, and congestion and hemorrhage of the

adrenals and kidneys. The changes in the adrenals were not remarkable in cases where the infected guinea pigs died after a long illness.

Microscopic sections of the kidneys stained with hematoxylin and eosin showed the capillary vessels, especially those of the cortex, to be enlarged and congested, with occasional hemorrhagic spots. The convoluted uriniferous tubules and glomeruli showed a degree of hyaline degeneration of the cells, as in acute nephritis. In the adrenals the capillary vessels were enlarged and congested, and small hemorrhagic spots were observed. The capillary vessels in the lungs and liver were also distended and congested.

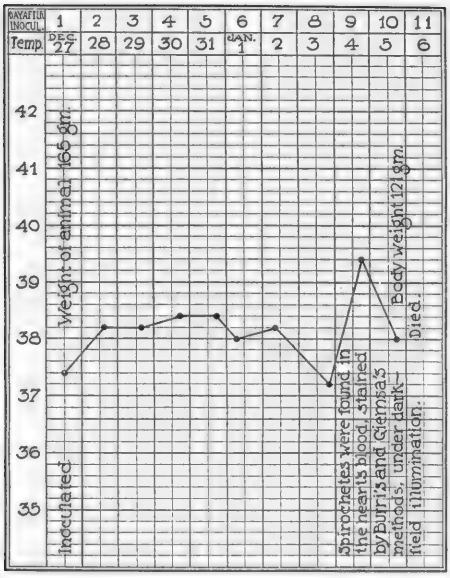
Further Inoculation Experiments.

When a guinea pig died of the fever, emulsions were made from the lymph and adrenal glands, brain substance, heart's blood, etc. The inoculation of these emulsions into the subcutaneous tissues or peritoneal cavities of fresh guinea pigs, always caused the death of the animals after giving rise to such symptoms as high temperature, swelling of the lymph glands, and a decrease in body weight. In these animals, which we called the first generation, the disease ran a shorter course than in the original generation. Some of the experiments are given below.

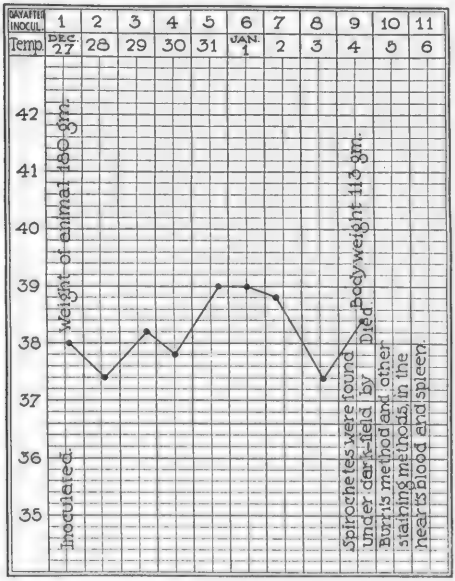
Guinea Pig 4, first generation, (Text-fig. 4) was inoculated on December 27, 1915, by injecting the heart's blood taken from Guinea Pig 3, original generation, intraperitoneally. At autopsy swelling and congestion of the inguinal, axillary, mesenteric, and retroperitoneal glands were found. No changes were observed in the peritoneal cavity where the inoculation had been made, but the changes in the kidneys and adrenals were typical. Spirochetes were found in the preparations made from the heart's blood stained with Burri's and Giemsa's solutions and also examined under dark-field illumination. Four fresh guinea pigs were inoculated with the heart's blood of this animal.

Guinea Pig 5, first generation, (Text-fig. 5) was inoculated subcutaneously with an emulsion made from the mesenteric lymph node of Guinea Pig 3, original generation, at the same time as Guinea Pig 4.

Autopsy.—The inguinal, axillary, and mesenteric lymph nodes were swollen and congested. The pathological changes of the kidneys and adrenals were typical. Spirochetes were found under dark-field illumination, by Burri's method and other staining methods, in the heart's blood and spleen.



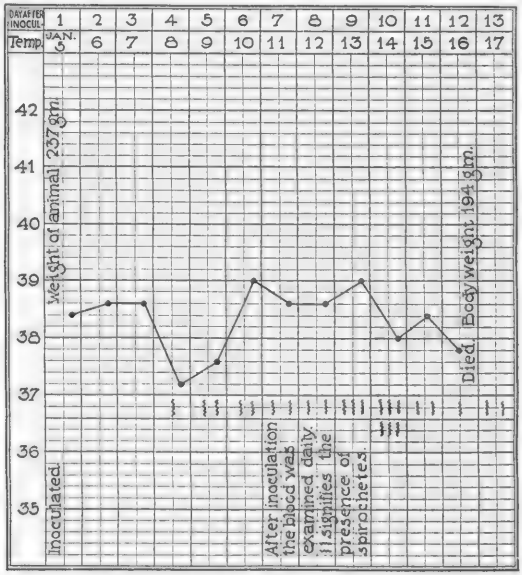
TEXT-FIG. 4. Temperature chart of Guinea Pig 4 (first generation).



TEXT-FIG. 5. Temperature chart of Guinea Pig 5 (first generation).

Further inoculation into mice, white rats, and guinea pigs gave positive results. Spirochetes were found in the heart's blood and films of the spleen under dark-field illumination, with Burri's and Giemsa's stains.

Guinea Pig 6, second generation, (Text-fig. 6) was one of the guinea pigs inoculated into the peritoneal cavity with the heart's blood of Guinea Pig 4 of the first generation. After inoculation we drew blood daily from the ear-lobes, examined it under dark-field illumination, and made stained preparations with Burri's and Giemsa's methods, and other stains. Spirochetes were present in all. The autopsy showed typical pathological changes.



TEXT-FIG. 6. Temperature chart of Guinea Pig 6 (second generation).

At autopsy the guinea pigs of the first generation showed swelling and congestion of the lymph glands and marked changes in the kidneys and the adrenals, as in the original generation.

The inoculation of fresh guinea pigs with the peripheral blood, heart's blood, and emulsion made from lymph glands taken from the guinea pigs of the first generation gave rise to the same symptoms

as in the first generation, and caused the death of the animals. The course of the disease and the result of the postmortem examination were similar to those of the first generation. We obtained the same result from the experiments with material from the animals of the 2nd, 3rd, 4th, and further generations, which indicates that there was no change in the pathogenicity of the spirochetes throughout the generations, which were many in number. It is thus seen that the guinea pig is highly susceptible to the disease.

Experiments on White Rats.

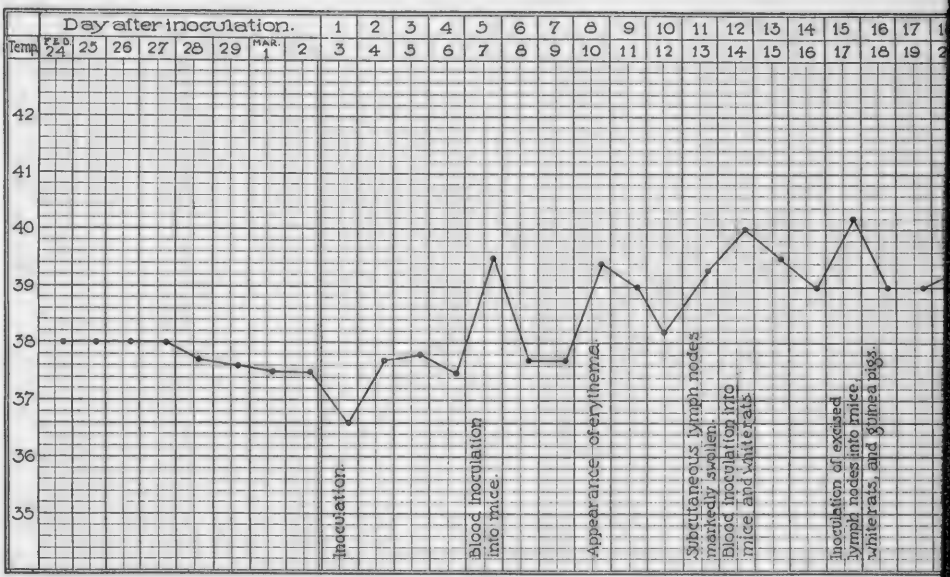
We inoculated a number of white rats subcutaneously or intraperitoneally with blood taken from an infected guinea pig. The inoculated animals showed no symptoms, but the spirochetes were found to multiply in their blood, as described below. The white rats did not die as a result of the inoculation, but spirochetes always developed in the blood of rats of the new generation. The inoculation into fresh guinea pigs of blood taken from the white rats of the new generation always gave rise to the usual symptoms, causing the death of the animals. In every instance spirochetes were found in the blood. Spirochetes were also found in the blood of mice inoculated with blood from the white rats.

We inoculated white rats with blood containing spirochetes from an infected guinea pig. After spirochetes were observed in the blood of the rats, the legs of two fresh guinea pigs were scratched with the rats' teeth until they bled slightly. One of the guinea pigs died several days later, but no spirochetes were found in the blood. The other guinea pig had fever on the 6th, 11th, 20th, and 25th days, and died on the 27th day.

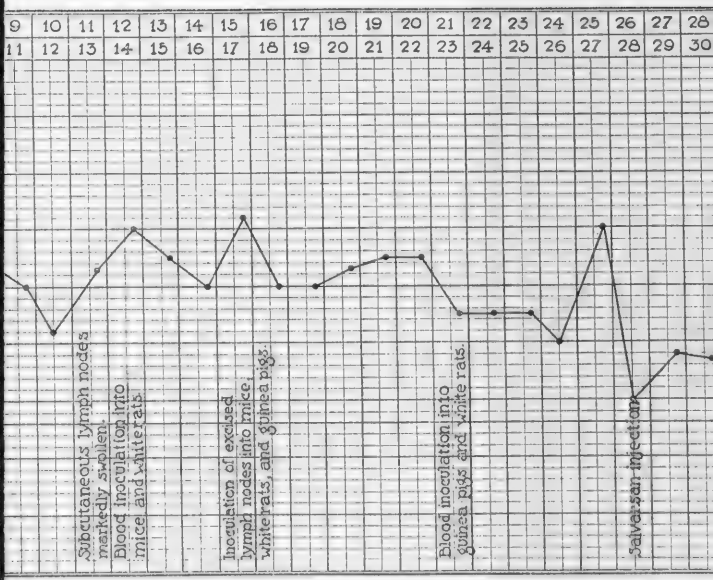
At autopsy the usual findings were present, though in less degree, and many spirochetes were observed in the heart's blood. We therefore concluded that the cause of infection existed in the mouths of experimentally infected white rats.

Experiments on White Mice.

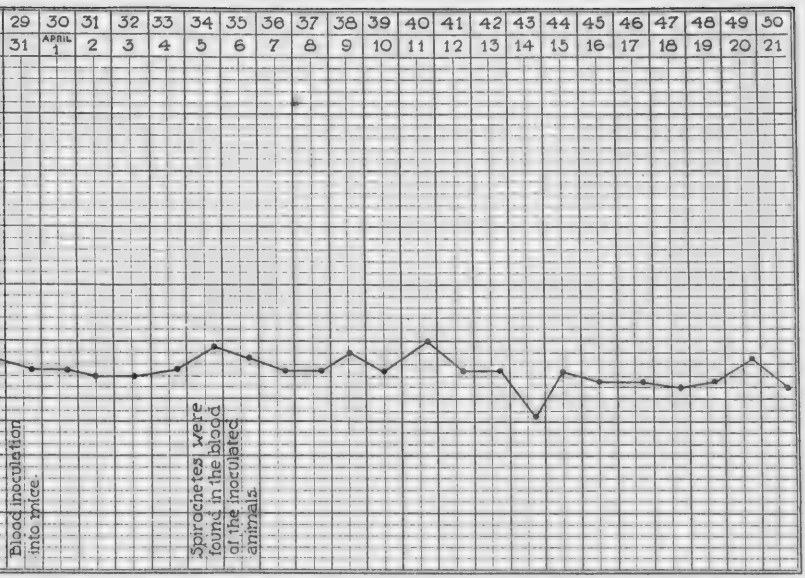
Spirochetes were also found in the blood of white mice inoculated with blood from an infected guinea pig. Only a few of the mice died



TEXT-FIG. 7. Temperature chart of Monkey 2 (*Pithecus rhesus* (Audeb


9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Subcutaneous lymph nodes markedly swollen.
Blood inoculation into mice and white rats.
Inoculation of excised lymph nodes into mice, white rats, and guinea pigs.
Blood inoculation into guinea pigs and white rats.
Salvarsan injection.

Monkey 2 (*Pithecus rhesus* (Audebert)) inoculated intraperitoneally with b


29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50
31 APRIL 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Blood inoculation into mice.
Spirochetes were found in the blood of the inoculated animals.

ood from a guinea pig with experimental rat-bite fever.

and no noteworthy macroscopic changes were found at autopsy. Guinea pigs inoculated with the blood of an infected mouse developed the usual symptoms and died. Spirochetes were always found in the blood. White rats inoculated with the blood of the mice also became infected. Many of the spirochetes in the blood of the mice had a large number of spirals.

Experiments on Rabbits.

We inoculated two rabbits, and no spirochetes were detected in their blood. The number of animals used, however, was too small to allow us to arrive at a definite conclusion.

Experiments on Monkeys.

We inoculated one *Pithecus irus* (F. Cuvier) and one *Pithecus rhesus* (Audebert) with blood from a guinea pig with experimental rat-bite fever (No. 7). The first monkey developed no symptoms of the disease, but spirochetes were found in the blood of mice and rats inoculated with the monkey's blood. In the *rhesus* monkey intraperitoneal inoculation caused a high temperature on the 5th day (the incubation period), and the lymph glands became swollen and were palpable. The swelling of one or two of the inguinal lymph nodes was especially marked. Erythema was also present. On the 8th day after the inoculation round red patches from 1 to 2 mm. in diameter appeared on the loins and the lower parts of the buttocks. These patches gradually increased in number, 40 to 50 being present on the 12th day. Most of them appeared close together on the skin, and finally coalesced into one another. The surface of the patches was slightly swollen, the red color fading on pressure. There were also some patches on the abdomen. The temperature rose every 2 or 3 days, and recurred six times in 25 days. The fever curve in the *rhesus* resembled that of human rat-bite fever (Text-fig. 7). No spirochetes were found in the films made almost every day from the peripheral blood or in the films from swollen lymph glands and excised lymph node tissue. Spirochetes were present, however, in rats and mice inoculated with the blood and emulsions made from excised glands.

Cultivation Experiments.

When an infected guinea pig of the original generation died, we made ordinary aerobic cultures of tissues taken from the site of the bite, the heart's blood, lymph glands, and fluids from the viscera and peritoneal cavity. In these cultures we sometimes found bacilli resembling *Bacillus coli* in form, movement, manner of staining, and pathogenicity. In our opinion, these contaminating bacilli in the cultures came from the animals. No such growth was noticed in other cases. We have not yet succeeded in making cultures from blood containing spirochetes.

Description of the Organism.

Localization of the Spirochetes.—The first spirochetes that we found were in Guinea Pig 1, bitten by a rat on June 18, 1915. The symptoms, course of the disease, and the postmortem examination indicated that the infection produced in the animal was a typical example of experimental rat-bite fever. Spirochetes were detected in the cortical capillaries and the parenchyma near the capsule in preparations made from excised tissue of the adrenals and stained by Levaditi's method. Spirochetes were observed later in the preserved adrenals of other guinea pigs. In the living animals they were demonstrated in the peripheral blood under dark-field illumination with Burri's and Giemsa's stains and with aniline dyes. At autopsy they were also found in cover-glass preparations of the heart's blood and spleen tissues. The spirochetes were present in all generations, but they were not so numerous as the recurrent spirochetes in the blood of infected mice. The largest number of spirochetes observed in blood films was five or six in one field (Zeiss, oc. 4, oil immersion $\frac{1}{12}$).

Time of the Appearance of the Spirochetes.—The symptoms were irregular in the original generation of guinea pigs, as stated above. Spirochetes were found in the peripheral blood only at the last stage of the disease, and before that none could be observed with any degree of accuracy. The animals of the first and later generations, however, displayed the usual symptoms, and spirochetes were observed in the peripheral blood 4 days, rarely 2 days, after inoculation. They gradually multiplied in the blood of guinea pigs, mice, and

white rats, reaching the highest numbers in 8, 10, or 12 days. In white rats and white mice, the spirochetes could be detected more than 2 months after the date of inoculation. Guinea pigs always died within 2 weeks after inoculation.

Movement.—In preparations made from the peripheral blood of experimental animals and examined under dark-field illumination, the spirochetes were seen to be transparent, and to possess an active progressive movement. As they passed swiftly out of the field, accurate observation of their morphological characteristics was impossible. They became sluggish after some time, and made spiral, rotating, or other movements. At this stage, the spirochete resembled a wood-louse in shape and appeared to consist of several joints of transverse lines. It was almost impossible to recognize the original shape of a spirochete, and its spiral form may be observed only at a much later period. The spirochetes were still moving actively in the blood 24 hours after the death of the animal.

Form.—Preparations made from the heart's blood of guinea pigs and stained according to Giemsa's method showed that the size of the spirochetes varied from 1.6 to 3 μ in length, 2 μ being the average size. The width was approximately from 0.4 to 0.5 μ. They tapered slightly at both ends. There were from two to six spirals or more, the average being two or three. Under dark-field illumination they were found to possess a flagellum at each end, which was filamentary and two or three times as long as the body. Sometimes the flagellum was present at one end only. It was clearly seen in preparations made by Burri's method (Fig. 1), or impregnated with silver according to Levaditi (Fig. 2). The undulating membrane has not yet been observed.

Staining of Spirochetes.—The spirochetes stain uniformly, easily absorbing the color, when dyed according to Giemsa's method (Fig. 3) or with aniline dyes, or with Löffler's methylene blue, aniline gentian violet, or carbol-fuchsin, and they are Gram-negative. When the number of spirochetes contained in the blood is very small, the blood should first be thickly smeared over the cover-glass, then treated with 1 to 2 per cent acetic acid by Koch's method, and finally dyed with gentian violet. In one instance two spirochetes apparently in the process of division seemed to be joined together (Fig. 3). We shall report more fully on the morphology later.

Experiments with Salvarsan.

Fourteen inoculated white rats, in the blood of which spirochetes were observed, were divided into three groups and given subcutaneous injections of salvarsan. The groups received 0.2, 0.1, and 0.06 gm., respectively, per kilo of body weight. Although many spirochetes were present in their blood before inoculation, none were detected on the 1st, 2nd, 3rd, 6th, and 10th days after inoculation. On the 17th day only a small number of spirochetes was found in one of the white rats into which 0.06 gm. per kilo had been injected, while in the control rats they were constantly present. We also obtained the same result by injecting into thirty-six mice a quantity of salvarsan varying from $\frac{1}{400}$ to $\frac{1}{1,000}$ gm. per 20 gm. of body weight. The details of this experiment are shown in Tables I and II.

TABLE I.

Injection of Salvarsan into White Rats.

No. of animal.	Weight.	Dose per kg.	Spirochetes before injection.	Spirochetes after injection. Day.							
				1	2	3	6	10	17	26	33
	gm.	*gm.*									
1	87	0.2	+++	−	−	−	−	−	−	−	−
2	78	0.2	+++	−	−	−	−	−	−	−	−
3	46	0.2	+++	−	−	−	−	−	−	−	−
4	53	0.2	+++	−	−	−	−	−	−	−	−
5	41	0.2	+++	−	−	−	−	−	−	−	−
6*	43	0.2	+++	++	+	+	+	+	+	+	+
7	43	0.2	+++	−	−	−	−	−	−	−	−
8	58	0.2	+++	−	−	−	−	−	−	−	−
9	172	0.1	+++	−	−	−	−	−	−	−	−
10	137	0.1	+++	−	−	−	−	−	−	−	−
11	59	0.1	+++	−	−	−	−	−	−	−	−
12	40	0.1	+++	−	−	−	−	−	−	−	−
13	115	0.06	+++	−	−	−	−	−	−	−	−
14	104	0.06	+++	−	−	−	−	−	+	−	−
15	77	−	+++	+++	+++	+++	+++	++	++	+	+†
16	51	−	+++	+++	+++	+++	+++	++	++	+	+†
17	40	−	+++	+++	+++	+++	+++	++	++	+	+†

* The dose of salvarsan was insufficient, owing to a technical mistake.

† Spontaneous decrease of spirochetes.

TABLE II.

Injection of Salvarsan into Mice.

	No. of animal.	Weight.	Dose per 20 gm.	Spirochetes before injection.	Spirochetes after injection. Day. 1	2	3	4	7	10	14	17	19
		gm.	*gm.*										
	1	15	$\frac{1}{400}$	+++	Died.								
	2	15	$\frac{1}{400}$	+++	−	−	−	−	−	−	−	−	+
	3	18	$\frac{1}{400}$	+++	−	−	−	−	−	−	−	−	+
	4	15	$\frac{1}{400}$	+++	−	−	−	−	−	−	−	−	+
	5	15	$\frac{1}{400}$	+++	−	−	−	−	−	−	−	−	+
	6	15	$\frac{1}{400}$	+++	−	−	−	−	−	−	−	−	+
	7	15	$\frac{1}{400}$	+++	−	−	−	−	−	−	+	−	+
	8	15	$\frac{1}{400}$	+++	−	−	−	−	−	−	−	−	+
	9	15	$\frac{1}{400}$	+++	−	−	−	−	Died.				
	10	15	$\frac{1}{600}$	+++	−	−	−	−	−	−	−	−	+
	11	15	$\frac{1}{600}$	+++	−	−	−	−	−	−	−	−	+
	12	17	$\frac{1}{600}$	+++	−	−	−	−	−	−	−	+	+
	13	15	$\frac{1}{600}$	+++	−	−	−	−	−	−	−	−	+
	14	17	$\frac{1}{600}$	+++	−	−	−	−	−	−	−	−	+
	15	19	$\frac{1}{600}$	+++	−	−	−	−	−	−	−	−	+
	16	15	$\frac{1}{600}$	+++	−	−	−	−	−	−	−	−	+
	17	19	$\frac{1}{600}$	+++	−	−	−	−	−	−	+	−	+
	18	15	$\frac{1}{600}$	+++	−	−	−	−	−	−	−	−	+
	19	15	$\frac{1}{800}$	+++	−	−	−	−	−	−	+	+	+
	20	17	$\frac{1}{800}$	+++	−	−	−	−	−	−	+	+	+
	21	15	$\frac{1}{800}$	+++	−	−	−	−	−	−	Died.		
	22	15	$\frac{1}{800}$	+++	−	−	−	−	−	−	+	+	+
	23	15	$\frac{1}{800}$	+++	−	−	−	−	−	−	−	+	+
	24	15	$\frac{1}{800}$	+++	−	−	−	−	−	−	−	+	+
	25	15	$\frac{1}{800}$	+++	−	−	−	−	−	−	+	+	+
	26	15	$\frac{1}{800}$	+++	−	−	−	−	−	−	−	−	+
	27	15	$\frac{1}{800}$	+++	−	−	−	−	−	+	+	+	+
	28	15	$\frac{1}{1000}$	+++	−	+	−	+	+	+	+	+	+
	29	17	$\frac{1}{1000}$	+++	−	+	+	+	+	+	+	+	+
	30	15	$\frac{1}{1000}$	+++	−	−	−	−	−	−	+	+	+
	31	17	$\frac{1}{1000}$	+++	−	−	−	−	−	−	−	+	+
	32	15	$\frac{1}{1000}$	+++	+	−	−	−	+	+	−	+	+
	33	15	$\frac{1}{1000}$	+++	+	−	−	−	−	−	+	+	+
	34	17	$\frac{1}{1000}$	+++	+	−	−	−	+	+	+	+	+
	35	18	$\frac{1}{1000}$	+++	+	−	−	−	+	+	+	+	+
	36	17	$\frac{1}{1000}$	+++	−	−	−	−	−	−	+	+	+
Controls.	37	15	−	+++	+++	−	++	++	+	+	+	−	+
	38	18	−	+++	+	Died.							
	39	13	−	+++	+++	+++	+	+	+	+	+	+	+
	40	17	−	+++	++	++	+	+	+	++	+	+	+
	41	15	−	+++	+++	+++	++	+	+	+	+	+	+
	42	17	−	+++	+++	+++	+	++	+	+	Died.		
	43	15	−	+++	++	++	+	++	++	++	+	+	+
	44	17	−	+++	+++	+++	+	+	++	++	+	+	+
	45	15	−	+++	++	++	+	+	+	+	+	+	+
	46	15	−	+++	+++	+++	+	+	+	+	+	+	+

Identification with Other Species.

Pathogenic and non-pathogenic spirochetes have been observed in many animals. We shall compare the various spirochetes with ours—except those which clearly differ in form—the animals in which they exist, and the results of experiments on animals.

(1) Wenyon's *Spirochæta muris*[9] is extremely motile. He attributes this to the flagella, though he does not claim to have proved it. The undulating membrane was not observed. The number of spirals varies between six and two according to the size of the spirochetes, which measure 6 to 7, or 3 to 4 μ in length and 0.2 μ in breadth. When inoculated into mice, they begin to appear in the blood in 5 or 6 days, and though few at first, they attain the greatest number about the 10th day and then decrease again in number. In 2 or 3 months they disappear entirely from the blood and at no period do they produce pathogenic symptoms in the mice.

Wenyon's spirochete and that found by us are similar in form, motion, the absence of pathogenicity for mice, and the manner of multiplication. In his experiment, however, guinea pigs did not become infected even with the whole blood of a mouse—a point on which Wenyon's spirochete differs from ours, since our spirochete invariably infects a guinea pig and can be seen in its blood. Wenyon further states that his spirochete can infect young rats but not full grown ones. Our spirochete, on the other hand, infects both young and adult rats.

(2) Breinl and Kinghorn's[10] *Spirochæta laverani* was found in a white mouse infected with *Trypanosoma dimorphon* sent by Laveran from the Pasteur Institute in Paris, and in the blood of two of the six wild mice caught in the neighborhood of the Liverpool School of Tropical Medicine. It is very motile, readily stains with the aniline dyes, and all the modifications of Romanowsky's method. It is generally short and round, but some are long, measuring between 1.8 and 3.75 μ in length and 0.1 and 0.2 μ in breadth. The number of spirals varies between two and four. Only a few of these parasites are found in the blood. It readily infects mice and rats, but gives rise to no pathogenic symptoms. Breinl and Kinghorn did not find many of the spirochetes in the blood, but could prove their existence in the peripheral blood 2 months after inoculation. They regard their spirochete as a distinct variety and have named it *Spirochæta laverani*. It seems to resemble our spirochete in form, movement, staining reaction, and its condition in mice and rats, but the result of the attempts to transmit it to guinea pigs is unknown.

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[9] Wenyon, C. M., *J. Hyg.*, 1906, vi, 580.

[10] Breinl, A., and Kinghorn, A., *Lancet*, 1906, ii, 651.

(3) Several investigators have found spirochetes in the cancer of mice. Borrel,[11] who was probably the first to do so, observed them in four mice suffering from primary cancers. After him Gaylord[12] examined forty-eight mice with cancer and more without cancer, chiefly by Levaditi's method, and observed numerous spirochetes. According to him, they stain readily by Levaditi's method, but not by Giemsa's; they are motile, but no flagella are seen; they vary between 2.5 and 7.8 μ in length, and have from four to thirteen spirals. Calkins[13] and Tyzzer[14] have reported the same spirochete.

Deetjen[15] likewise made a study of mouse cancer and observed a species of highly motile spirochetes. It stained readily by Giemsa's method, measured from 1.5 to 5 μ, and had from one to five spirals and a flagellum about 3.0 μ long at each end. He states that the flagella occasionally failed to stain except by Levaditi's method.

Löwenthal[16] observed small spirochetes from 2.6 to 6 μ in length and 0.25 or 0.2 μ in breadth with four to twelve spirals in ulcerated cancer from human beings and dogs. They stained readily; no flagella and no undulating membranes were seen, but Löwenthal believes that they are provided with the latter. He called this species *Spirochæta microgyrata*.

It is difficult to identify the species of spirochetes, since the size of a spirochete or the number of its spirals varies according to the method of treatment. In experiments on animals, too, different results do not prove conclusively the difference of species. Deetjen, however, thinks that his spirochete is identical with Wenyon's *Spirochæta muris* and Breinl and Kinghorn's *Spirochæta laverani*, and that although it is difficult to identify the spirochetes found by Borrel, and Gaylord, and Tyzzer with his, they are undoubtedly similar species. Wenyon, on the other hand, considers that his species is distinct from Carter's.[17]

We have examined the blood of a large number of normal mice and white rats, treated with acetic acid, but have not found a single spirochete. We have not, however, examined the cancer of mice; we cannot, therefore, attempt to identify our spirochete with those observed by the investigators mentioned above.

[11] Borrel, A., *Compt. rend. Soc. biol.*, 1905, lviii, 770.
[12] Gaylord, *Ann. Rep. Cancer Lab. New York State Dept. Health*, 1907, viii, 34.
[13] Calkins, G. N., and Clowes, G., *J. Infect. Dis.*, 1905, ii, 555.
[14] Tyzzer, E. E., *Proc. Soc. Exp. Biol. and Med.*, 1906–07, iv, 85.
[15] Deetjen, H., *Münch. med. Woch.*, 1908, lv, 1167.
[16] Löwenthal, W., *Berl. klin. Woch.*, 1906, xliii, 283.
[17] Carter, V., *Sc. Mem., Med. Officers Army of India*, 1887, iii, 45.

We should mention also the spirochetes in rats found by Carter[17] (1887), MacNeal[18] (1907), and Mezinescu[19] (1909). As we have stated, Wenyon finds it impossible to identify his spirochete with Carter's, and Breinl and Kinghorn also believe that Carter's spirochete, which is larger and has more spirals, must be different from Wenyon's. Mühlens,[20] however, is inclined to believe that they all belong to the same species. In only one instance, among all the normal rats whose blood we have examined, did we find spirochetes similar in form. Since we made no further test, we cannot, however, go into the details of the subject. The spirochetes found by MacNeal, Mezinescu, and others do not give rise to pathogenic symptoms in the animals into which they are inoculated. However, the spirochetes of these investigators and the spirochete found by us appear from inoculation tests to be similar in form. As their tests were not the same as ours, it is difficult to compare the spirochetes. Unlike the investigators referred to above, we first undertook the bite experiments and observed the course of the infection after the bite and the pathological appearance at autopsy, and finally the pathogenicity of the virus for the guinea pig and monkey. The favorable reaction with salvarsan seems also to be important.

As we have stated, we always observed traces of acute changes in the kidneys and adrenals of the guinea pigs bitten by rats and of guinea pigs inoculated from the original generation. We are, however, at present unable to compare experimental and human rat-bite fever since only two reports on the anatomical view of the latter have appeared. Blake believes that Schottmüller's *Streptothrix muris ratti* is the causative agent of the disease. His diagnosis was "acute ulcerative endocarditis; subacute myocarditis; subacute interstitial hepatitis; subacute glomerular and interstitial nephritis, subacute perivascular exudate of adrenals; infarcts of spleen and kidney; congestion, hemorrhage, and edema of lungs; atrophic leiomyoma of the uterus."[21] Blake's report, however, affords us little help in our anatomical study of guinea pigs, inasmuch as we cannot be certain whether the abnormalities in the kidneys and the adrenals were due to rat-bite fever or to a streptothrix. The other report, which is by Miura and Toriyama,

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[18] MacNeal, W. J., *Proc. Soc. Exp. Biol. and Med.*, 1907, iv, 125.

[19] Mezinescu, D., *Compt. rend. Soc. biol.*, 1909, lxvi, 58.

[20] Mühlens, P., in Kolle, W., and von Wassermann, A., Handbuch der pathogenen Mikroorganismen, Jena, 2nd edition, 1913, vii, 921.

[21] Blake, *J. Exp. Med.*, 1916, xxiii, 47.

does not mention the condition of the kidneys and adrenals. Crohn[22] described fifty-two cases of human rat-bite fever, and although he did not have the result of the examination of urine in every case, he enumerates nine cases of nephritis. From this it seems to be evident that human rat-bite fever also frequently affects the kidneys.

SUMMARY.

1. We have confirmed Ogata's results in experimental rat-bite fever caused by the bite of rats.

2. In our experiments with guinea pigs, swelling and congestion of the bitten parts, swelling of the subcutaneous lymph nodes, fever, and loss of weight were the typical symptoms. The progress of the fever was not so regular as in human cases, but we find records in the literature of patients who showed irregular fever types or were afebrile. The chief points that we noted in the anatomical view of the guinea pigs were swelling and congestion of the lymph gland system and acute changes in the adrenals and kidneys.

3. If an emulsion made from the lymph glands, cerebral substance, or the adrenals, or the heart's blood of a guinea pig of the original generation is inoculated subcutaneously or intraperitoneally into a fresh guinea pig, the animal invariably dies with the usual symptoms of fever and swelling of the lymph glands. The anatomical changes in this case were the same as those of the original guinea pig, except that the course was shorter and more regular. The same result was observed in further generations. No change was observed in pathogenicity, and in the guinea pigs of the original and further generations a species of spirochete as the causative agent was always observed. The incubation period in the original generation was from 1 to 2 weeks, and in further generations about 1 week.

4. When a mouse or white rat was inoculated, spirochetes always appeared in the peripheral blood, but no other symptoms developed. When peripheral blood drawn from a mouse thus treated was inoculated into a fresh mouse or a fresh white rat and peripheral blood drawn from the mouse and rat was inoculated into a fresh guinea pig, they all became infected and the guinea pigs always died. Thus we found that rats and mice are media but not victims of the disease, while guinea pigs are both media and victims of it.

[22] Crohn, B. B., *Arch. Int. Med.*, 1915, xv, 1014.

5. In the *rhesus* monkey on which we made our experiment we witnessed a process similar to that of human rat-bite fever, and our spirochetes were observed in other animals into which blood drawn from the monkey was inoculated.

6. In the original animals spirochetes were seen chiefly toward the end of the process and the conditions as to the period previous to it are not yet clearly known. In further generations of all the animals we used, spirochetes were found in the peripheral blood 4 or 5 days after inoculation, and gradually multiplied until the greatest number was reached about the 10th day after inoculation. They then began to decrease; yet spirochetes could be observed over 2 months later.

7. We have found spirochetes chiefly in the adrenals of the animals by Levaditi's method, but have not yet ascertained their distribution in other organs.

8. Our spirochete is short, round, and highly motile; it stains readily, and has few spirals. We have not yet observed an undulating membrane, but have seen what we believe to be a flagellum at each end.

9. The identification of our spirochete with other species must be left for further study. The spirochete which Futaki, Takaki, Taniguchi, and Osumi[8] found in two patients with rat-bite fever, seems to differ from ours in form.

10. Spirochetes disappear from the blood of the animals as a result of the injection of salvarsan, thus indicating that the spirochete is arsenotropic.

In conclusion we wish to express our indebtedness to Professor M. Ogata, to Professor Aoyama, Director of the Imperial Institute for Infectious Diseases, and to Professor Hayashi and Drs. Miyagawa and Mitamura, for their valuable assistance.

EXPLANATION OF PLATES.

PLATE 11.

FIG. 1. Spirochetes in the blood of a guinea pig infected with experimental rat-bite fever. Stained by Burri's method.

FIG. 2. A spirochete in a section of the adrenal of the same guinea pig as in Fig. 1. Stained by Levaditi's method.

FIG. 3. Spirochetes in the blood of a guinea pig infected with experimental rat-bite fever. Giemsa's stain. Zeiss, oc. 4, obj. $\frac{1}{12}$ oil immersion.

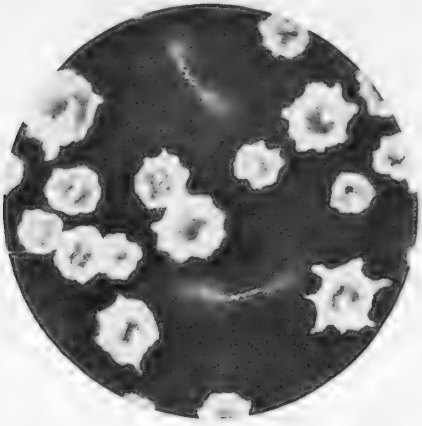

FIG. 1.

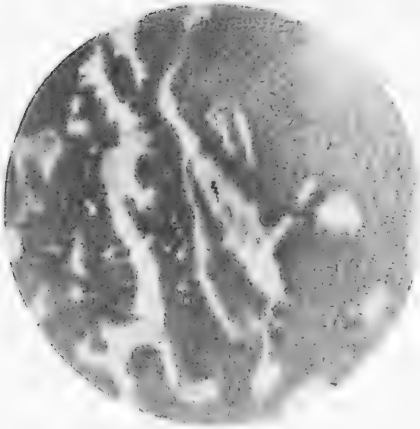

FIG. 2.

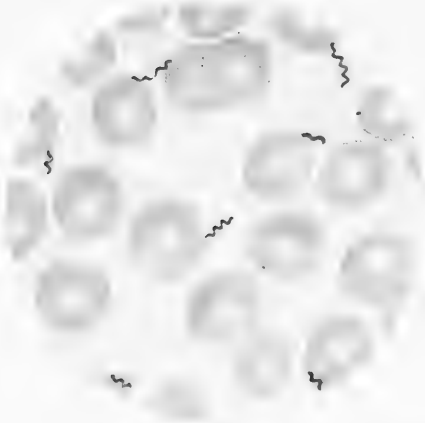

FIG. 3.

(Ishiwara, Ohtawara, and Tamura: Experimental Rat-Bite Fever.)

THE ACTION OF DIGITALIS IN PNEUMONIA.

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PLATE 12.

(Received for publication, October 17, 1916.)

Digitalis has been used for many years in the treatment of pneumonia but there is still discussion as to whether its use is advantageous. A decision has been difficult because the difference between action as such and beneficial action has not been sharply drawn. We show in this paper that action on the heart by digitalis takes place in pneumonia, and also that its action under certain circumstances is beneficial.

HISTORICAL.

Schmiedeberg (1) has given the early history of the use of digitalis in pneumonia. It was recommended by Ferrier (1799) for the purpose of slowing the heart rate instead of resorting to phlebotomy. Currie (1804) preferred it to cold water treatment in cerebral, cardiac, and pulmonary inflammations. Cuming (1804) gave it after phlebotomy. Then, says Schmiedeberg, its use was almost forgotten. Traube (1850), who learned the value of digitalis in Schoenlein's clinic, reintroduced it. Because it slowed the pulse Traube believed that it was an antipyretic but, as von Leyden showed, he failed to recognize the fact that if the pulse rate was slowed to the point of lowering temperature, this result was due to collapse. At this point Schmiedeberg leaves the history. He believed that giving digitalis in pneumonia was a matter still open to investigation.

More recently clinicians have been divided in opinion on its usefulness in this disease. Gibson (2) is doubtful; and Mackenzie (3) says: "I have never seen much good follow the administration of digitalis in acute febrile states. The factors exciting the heart, such as high temperature, toxins, or the invasion of the heart by specific organisms, exert an influence over the heart which the digitalis can not overcome." Lauder Brunton (4), in discussing the effect of temperature (not fever) on the action of drugs goes so far as to say: "Perhaps one of the most marked examples of this is digitalis, which at a high temperature completely loses its power of slowing the heart through the vagus." In the United States, certain authors, as Hare (5), share Brunton's view. Others, like

Elsner (6), enumerate specific indications for giving digitalis. In Germany, on the other hand, digitalis is more commonly administered now. Romberg (7) gives it to all pneumonia patients suffering from heart disease as soon as the diagnosis is made. Meyer (8) reports that it is constantly used in Krehl's clinic with gratifying results. Fraenkel (9) gives large doses (3 to 4 gm. a day for the first 3 days) in accordance with the Traube tradition.

An effort has been made to ascertain by experiment whether in the presence of fever any agents are at work which interfere with the action of digitalis. All these experiments have been employed for the purpose of determining whether heat interferes with the action of the drug. Trendelenburg (10), Weizsäcker (11), Gunn (12), and von Issekutz (13) have all performed such experiments either on rabbit or frog hearts, with digitalis or amorphous or crystalline strophanthine preparations. The result of all these efforts has been to show that by elevating the temperature of the perfusion fluid containing the digitalis body, the end-point of the reaction appears earlier than in the controls; that, in other words, elevation of temperature facilitates the action of digitalis bodies.

In the experiments performed by Jamieson (14) on dogs and cats, on the other hand, the problem investigated was whether pneumonic infection had an influence on the action of digitalis other than that exerted by temperature alone. He could find no difference in the minimal lethal dose of crystalline *g*-strophanthine in normal animals and in infected animals when the infection was at its height. He concluded, therefore, that there was nothing in the nature of the infection to interfere with the action of the drug. This conclusion does not, however, mean that the heart in pneumonia is an altogether unchanged and intact organ. Newburgh and Porter (15), for instance, have shown that it must be altered, for the hearts of animals infected with the Friedländer bacillus continue to beat when nourished with pneumonic blood, whereas such blood quickly poisons control hearts from non-infected animals. It is clear, however, from the results of Jamieson's work that, whatever the poison to which pneumonic hearts have accommodated themselves, its nature is not such as to interfere with digitalis activity.

Method.

Our observations have been carried out on 105 cases of pneumonia treated in the Hospital of The Rockefeller Institute. Digitalis was given to 49 patients; the other 56 received no digitalis and served as controls. The drug was usually given by mouth in the form of tablets of digipuratum, each tablet containing the equivalent of 0.1 gm. of the powdered leaves. The daily dose was usually 0.4 gm. Electrocardiograms were made of all the patients. In the patients to whom digitalis was administered the curves were made frequently, usually once, or oftener, each day. At the beginning of the study,

TABLE I.

	Control. No digitalis.			With digitalis.				Total available.	Total.
	Repeated records.	Single records.	Total.	Repeated records. Available.	Repeated records. Not available.	Single records. Not available.	Totals.		
Recovered.....	41	8	49	28	3	—	31	77	80
Died.....	4	3	7 (12.5%)	11 (28.2%)	3	4	18 (36.7%)	18 (18.9%)	25
Total.....	45	11	56	39	6	4	49	95	105

Detail of Cases.

	Fever. Recovered.	Fever. Died.	No fever. Recovered.	Fever. Recovered.	Fever. Died.	No fever. Recovered.	Per cent. Fever and no fever.	Per cent. Fever.	Fever. Recovered.	Fever. Died.	No fever. Recovered.	Fever. Recovered.	Fever. Died.	No fever. Recovered.	Fever. Recovered.	Fever. Died.	No fever. Recovered.	Total. Per cent. Fever and no fever.	Total. Per cent. Fever.	Available. Per cent. Fever and no fever.	Available. Per cent. Fever.	
Changes P-R and T.	2						3.5	4.0	21	7	2							61.2	60.8	76.9	77.77	97.2
" T alone.	3	1	4			1	16.0	8.0	3	3		1	2			2		22.4	23.9	15.3	16.66	
" P-R "									1		1							4.08	2.1	5.1	2.77	
" neither.	32	3		6	3	1	80.3	88.0		1		2	1			2		12.2	13.04	2.5	2.77	
Totals.....	37	4	4	6	3	2	56	50	25	11	3	3	3			4		49	46	39	36	
	45			11					39			6			4							

however, daily electrocardiograms were not made because the importance of frequent observations for the purpose of this investigation was not appreciated.

We have utilized as criteria for judging the action of digitalis the effect upon the length of the auriculoventricular interval (the P–R time) and the effect on the T wave of the electrocardiogram. The length of the auriculoventricular interval was calculated from electrocardiograms. The changes in the T wave for which we looked were the ones described in an earlier paper (16). In certain cases the effect of digitalis on the rate of the ventricles when the auricles were fibrillating has been an important additional criterion.

RESULTS.

In general the criteria we employed permitted us to judge satisfactorily whether digitalis was acting. We found that the signs appeared after the same amount had been given and following the same length of time in which these signs appeared in the non-febrile cases originally studied. When no digitalis was given the signs did not appear.

In 45 of the 56 control cases (Table I) we were able to make two or more observations (repeated records), while in 11 others only single electrocardiograms (single records) were obtained.

Of the 49 patients to whom digitalis was given, 39 are represented by two or more electrocardiograms (repeated records). They are grouped as available because they received amounts of digitalis sufficient to influence the curve. 6 patients are classified as unavailable because they took total amounts of digitalis (from 0.3 to 0.6 gm.) which as a matter of experience are regarded as too small to influence the curve. The remaining 4, on whom only single observations were made, are rejected for the same reason. The doses given to them were 0.7, 0.1, 0.4, and 0.2 gm.[1] The amount regarded as sufficient to produce the changes we looked for was fixed at 0.8 gm. No one showed changes after taking an amount less than this, except a single patient to whom the drug may have been given before admission to the hospital.

[1] In the last three cases the amounts given are approximate.

We distinguish further, both in the control and in the digitalis groups, between those patients who showed alterations in the curves during fever and those in whom defervescence had already taken place when the alterations were found. This distinction is necessary because what we wish to ascertain is the action of digitalis during the continuance of fever. The cases have been further subdivided into (1) those which showed changes both in P–R time (auriculoventricular interval) and in the T wave; (2) those which showed changes in the T wave alone; (3) those which showed changes in the P–R time alone; and (4) those which showed changes neither in the P–R time nor in the T wave. We base our percentage figures, therefore, on 50 cases of pneumonia who received no digitalis during the febrile period of the disease; and on 36 to whom digitalis was administered, and who took a sufficient amount of the drug.

Of the 50 control patients, 88 per cent (44 cases) showed no change in either P–R time or in the T wave; 8 per cent (4 cases) showed changes in the T wave alone; 4 per cent (2 cases) showed changes in both the P–R time and in the T wave. Of the 36 patients to whom digitalis was given, on the other hand, 77.7 per cent (28 cases) showed changes both in the P–R time and in the T waves; 16.6 per cent (6 cases) showed changes in the T wave alone; 2.7 per cent (1 case) showed changes in the P–R time alone; and 2.7 per cent (1 case) showed changes in neither the P–R time nor in the T wave. The results show briefly, therefore, that during the febrile period 88 per cent of the control cases showed no change in the electrocardiogram; while 97.2 per cent of the cases to whom digitalis was given, did show changes.

Besides the effect on conduction and on the form of the electrocardiogram (T wave), we were able to study the action of digitalis on the rate of the ventricles when the auricles were fibrillating. In five separate instances when this rhythm set in, digitalis reduced the resulting high ventricular rate while fever was present. These cases will be described in detail in a separate communication.

The facts given permit us to conclude that digitalis acts in pneumonia.

Exceptions.—The cases here described are those which were not included in the two percentage figures (88 and 97.2 per cent) just given. These include

6 control cases during fever, 5 others after defervescence, and 4 (1 febrile and 3 non-febrile) available and 10 non-available cases to which digitalis was given. We shall take up first the 6 control cases during fever. 2 showed changes both in the P–R time and in the T wave. It cannot be definitely stated that these changes occurred spontaneously, because it is not known whether digitalis was given outside the hospital. 4 other control patients showed changes in the T wave alone. 2 of these (a mother and her son) entered the hospital rather late in the course of the disease (the son on the 5th day). The 3rd showed the change only in the first curve; the 4th in a curve a week before death, on the 4th day in the hospital. In addition to these, 5 other control patients showed changes in the T wave in the period after defervescence. We can offer no explanation for their occurrence. The point of immediate interest lies in the fact that when no digitalis was given, the occurrence of electrocardiographic changes during fever was infrequent (6 in 49 cases).

We take next the single patient to whom a sufficient amount of digitalis was given and in whose curves during the febrile period the expected alterations did not take place. This patient died. Three curves were made in the 3 days he was in the hospital, and all showed that the T wave in Lead I was inverted. Whether this was normal for him, or whether it represented a change is unknown. No further change in his curves was observed, and we classify him accordingly as having shown none. In the other three available cases it is necessary only to notice that the changes occurred after the fever ceased.

We consider finally the 10 unavailable cases to which digitalis was given during fever. 5 showed changes in the T wave alone; 5 showed changes in neither. In the latter 5 no change is expected (they had taken insufficient amounts); they require no further discussion. The other 5 took digitalis, but in insufficient amounts. Of these, 1 recovered and 4 died. The one who recovered took 0.5 gm. before the electrocardiogram changed. After convalescence his T wave again became upright. The 4 who died took, respectively, 0.6 gm., 0.5 gm., 0.2 gm., and the last probably less than 0.5 gm. (The time when the electrocardiogram was taken is not recorded.) Whether doses so small caused the change must remain undecided. We doubt that the drug can have been the cause. In these 5 cases to which digitalis was given and in the 6 (febrile) control cases, then, changes took place when no digitalis had been administered, at least in the hospital, or when it was given in amounts insufficient to satisfy one that digitalis was responsible for the change.

Although our report deals with the changes which occur primarily during fever, we have included in separate columns in the table those cases in which changes took place in the postfebrile period, and we have given the percentages of each variety of change in the total of febrile and non-febrile cases. In the cases to which digitalis was given the total available and the total non-available cases are given separately. The general interpretation we draw from our observations is not altered by this division. The conclusion that digitalis acts on the febrile heart in pneumonia is, of course, better seen when only the available cases in each group are considered.

DISCUSSION.

Auriculoventricular Conduction (P–R) Time.—The effect of pneumonia or the intoxication of pneumonia on heart muscle has generally been supposed to bring about an increase in the difficulty of auriculoventricular conduction. A number of cases have indeed been reported in which delay in, or block to the passage of impulses has occurred. But in the 50 control cases on which we report, it is striking that a significant increase in conduction did not occur. On the contrary, we observed an opposite tendency with equal frequency. A decrease in the conduction time was observed in 11 cases (Table II). The decrease was, however, slight; it was usually 0.02 to 0.03 second, twice 0.04 second, once 0.05 second, and once 0.06 second. When

TABLE II.

Hospital No.	P-R time.
2,315	Fell from 0.16—0.14 = 0.02 sec.
2,309	" " 0.16—0.13 = 0.03 "
2,270	" " 0.17—0.14 = 0.03 "
2,276	" " 0.20—0.17 = 0.03 "
2,325	" " 0.17—0.14 = 0.03 "
588	" " 0.18—0.15 = 0.03 "
2,187	" " 0.16—0.13 = 0.03 "
2,286	" " 0.17—0.13 = 0.04 "
2,202	" " 0.18—0.14 = 0.04 "
2,195	" " 0.18—0.13 = 0.05 "
2,269	" " 0.21—0.15 = 0.06 "

lengthening took place, as it did in 6 patients, the change occurred within limits as narrow (Table III) and did not extend beyond 0.3 second.

TABLE III.

Hospital No.	P-R time.
2,335	Rose from 0.15—0.17 = 0.02 sec. during fever.
2,296	" " 0.14—0.16 = 0.02 " " "
2,344	" " 0.14—0.17 = 0.03 " " "
2,184	" " 0.17—0.20 = 0.03 " " "
2,216	" " 0.13—0.16 = 0.03 " after "
2,168	" " 0.15—0.18 = 0.03 " " "

The absence of lengthening in conduction leading to heart block in this series[2] is the more striking on account of the fact that we are taught to look for its occurrence in the course of infectious fevers. In our control cases, as we have shown, it did not take place.[3] In the cases to which digitalis was given, on the other hand, there was with only three exceptions an increase of 0.04 second or more. In seven instances the rise led to block.

It is conceivable that delayed conduction in patients with pneumonia may occur simply as the result of the increased rate of the pacemaker; and second, that it may be a direct effect of the intoxication due to pneumonia itself. As far as the effect of increased rate is concerned, Lewis and Cotton (17) have shown satisfactorily that when normal individuals exercise, no lesion of the junctional tissues being present, the hearts respond by an increase in rate and a decrease in the auriculoventricular interval of 0.01 to 0.03 second. In our patients the conduction interval tended, indeed, during the height of the fever, to be lessened. This may have been due to the elevation in rate. When lengthening occurred it was distinctly within the range found in normal hearts. The influence which the intoxication of pneumonia exerts on this function will be discussed later.

A reasonable explanation for the failure of a marked change in conduction to occur spontaneously may be found in a consideration of the histological pathology of pneumonia. The infections which have an influence on auriculoventricular conduction are acute rheumatism, diphtheria, typhoid fever, syphilis, and occasionally influenza. The mechanism which brings this effect about in these diseases probably does not exist in pneumonia. In the other infections mentioned, definite anatomical changes may take place, as, for instance, focal necroses in typhoid fever; infiltrating and destructive

[2] In another series of patients, now being studied, an irregularity occurred in the postfebrile period in one case caused by premature auricular beats, which were blocked. These occurred during the systole of the preceding ventricle. The ventricle may have been in its refractory period. The conduction time was uniformly 0.14 second. Incomplete block in the ordinary sense was therefore absent.

[3] The conduction changes reported by others did not, except rarely, occur until a sufficient dose of digitalis had been administered. This subject will be discussed in a later paper.

inflammatory invasions in rheumatism and syphilis; fatty, granular, and waxy degeneration of the muscle and interstitial round cell infiltration in diphtheria. Any one of these alterations may involve the conduction pathway and may terminate in functional disturbance. Of the pathological histology of the heart in pneumonia there is still insufficient knowledge, but it does not appear that extensive alterations occur.

Hearts from pneumonia patients have been described by Romberg, Meyer, Aschoff and Tawara, Eyselein, Zadek, Thorel, Fraenkel, and Liebmann. In a recent review of the literature, Thorel[4] (18) says: that from the point of view of pathological anatomy the changes in the heart in pneumonia are insignificant. Serious myodegenerations do not occur. Fatty degeneration is occasionally seen in older individuals. Romberg and his associates (19), in experiments on animals, were struck by the absence even of a slight effect on the behavior of the heart in pneumococcal infections. They saw only small and unimportant changes in the heart muscle.[5] Zadek (20) found no lesion in one case and myodegeneration in another case (the second had slight albuminuria) dying late in convalescence. Aschoff and Tawara (21) examined five cases and could find no myocardial process or extensive specific parenchymatous change. Liebmann (22) in a more than ordinarily careful examination of eleven hearts, found lesions in only three, but in these three he found inflammatory infiltration. Such lesions in suitable locations might cause disturbances in rhythm. Eyselein (23) examined twenty-three cases; nineteen hearts showed no particular changes, four showed some fatty change, and three of these an advanced degree of this alteration (Table IV).

TABLE IV.

Author.	Total.	Lesions.
Zadek	2 cases.	1 case.
Aschoff and Tawara	5 "	0 cases.
Liebmann	11 "	3 "
Eyselein	23 "	4 "
	41 "	8 "

Anatomical lesions are, according to these investigations, infrequent and on the whole insignificant. This conclusion strengthens the belief, formed by clinical observation, that where moderately

[4] Thorel (18), p. 432.

[5] Romberg, Pässler, Bruhns, and Müller (19), p. 713.

lengthened conduction occurs, it is not due to an anatomical cause. Whatever the nature of the agent, it is clear that its action is not uniform, for within the narrow limits in which changes in our series took place, they were, as we have shown, in the direction both of lengthening and shortening.

T Wave.—In our earlier paper (16) we suggested that the occurrence of the T wave phenomenon was probably explained by the fact that since "cardiac action currents depend on electrical changes as an expression of muscular activity, then the changes in the T wave must be attributed to an alteration in muscular state under the influence of the drug" (digitalis). We pointed out at the same time that the change in the T wave we were describing was not specific for digitalis, but that it occurred under the influence of muscarin, under the alteration of physical states (as the application to the heart of heat and cold), and as the result of stimulating the vagus nerve by interrupted electrical currents. There is a temptation to add the intoxicating substance in pneumonia to the agents able to cause it, in view of the fact that a number of untreated cases showed a change in the T wave. But we do not include it because the change occurs so infrequently and because of the possibility that patients may have been given digitalis before being brought to the hospital. Furthermore, the T wave in nine control cases suffered no decrease in amplitude, but actually increased distinctly (Fig. 1). When to these considerations is added the fact that the change occurred in five patients after defervescence or after the crisis, then it becomes improbable that the intoxicating agent in pneumonia possesses this influence. What circumstance is responsible for the unexpected changes which we found we are not able to decide.

We shall report next on the dose required, and the order in which the effects on the T wave and on conduction take place. It is impossible to decide on the smallest efficient dose. That was possible in the non-febrile cases described in our earlier report, where there was no complicating intoxication, and where one could be sure that no digitalis had been given before treatment. The dose we regarded as effective was about 1 gm. To the pneumonia cases we have given almost uniformly 0.4 gm. in 24 hours. The largest total doses we gave were 4.45 gm. (1 patient), 3.8 gm. (1 patient), 3.3. gm. (1 pa-

tient), 3 gm. (1 patient), and 2.9 gm. (1 patient). The other 44 patients took 2.5 gm. or less.

We found in the group of available cases that alterations in the T wave alone were observed in 16.6 per cent, and changes in the P–R time alone in only 2.7 per cent. Where only one of the changes takes place, that change accordingly is more likely to affect the T wave. But where both alterations were observed (28 cases, 77.7 per cent) we found that they occurred simultaneously in 15 patients, that the T wave was altered with smaller doses in 8 patients, and that the P–R interval lengthened earlier in 5 (Table V).

TABLE V.

Hospital No.	Change in P-R time.	Change in T wave.
	gm.	*gm.*
2,338	After 0.9	After 0 (−T before digitalis.)
2,381	" 1.8	" 0.8
2,208	" 1.2	" 0.9
2,277	" 3.4	" 1.1
1,991	" 1.7	" 1.3
2,247	" 3.0	" 1.5
1,858	" 2.4	" 1.6
2,287	" 2.6	" 2.3
2,413*	After 0.4	After 0.8
2,284	" 0.8	" 1.2 (No fever.)
2,222	" 1.1	" 1.5
2,175	" 1.3	" 1.5
2,399	" 1.3	" 1.7

* This patient probably was given digitalis before admission to the hospital.

We conclude, therefore, that digitalis alters the T wave oftener and sooner than it does the P–R time. This result is in agreement with that found formerly by us when the results of giving digitalis to patients suffering from heart disease were reported. There, in all but five instances the changes in the T wave occurred before the change in conduction. In the present series, in all but five cases, the change in the T wave occurred at the same time with, or before the change in P–R time. As a guide, therefore, to giving digitalis to patients suffering from pneumonia, we ascribe a distinct value to the use of the T wave as a sign.

Method of Selection.—The frequency with which changes in P–R time and in the T wave occurred in the digitalis group might lead to the belief that, since in this group the mortality was high, the changes were due to approaching death. In other words, the great number of electrocardiographic changes in the cases to which digitalis was given may have been due either to the method of selection or to the influence on the heart exercised by the mechanism of approaching death.

The most satisfactory method of selection consists, obviously, in studying alternately control and treated cases. We did not adopt this method because we were unwilling to withhold digitalis from patients who were seriously ill. In the early cases, indeed, it was only the severe ones that were treated. They show, consequently, a larger number of deaths than would ordinarily be expected. The mortality of the control cases was low (Table I), 12.5 per cent (7 in 56 cases), but of the available treated cases it was more than twice as great, 28.2 per cent (11 in 39 cases). Of the untreated and available treated cases it was 18.9 per cent (18 in 95 cases). The average percentage of mortality in the hospital covering the period in which these cases were studied was 27.8 per cent (Table VI). That the

TABLE VI.

Year.	No. of cases examined.	Received digitalis.	Died.		Hospital mortality.
			No.	Per cent.	
		per cent			*per cent*
1911–12	6	50	4	66.6	47.6
1912–13	16	50	6	37.5	17.3
1913–14	19	68.4	8	42.1	20.2
1914–15	82	56.9	21	25.6	26.1
	123	56.3	39	42.9	27.8

mortality of the treated cases is high on account of the method of selection is suggested by the fact that in the year 1914–15, when we studied all the cases (with seven exceptions), we had the lowest mortality (25.6 per cent), whereas in the year 1911–12, when we studied only severe cases, the mortality was 66.6 per cent. The mortality of all the patients treated in the hospital during the same

TABLE VII.

Relation of Time of Death to Time of Making Electrocardiogram.

Case No.	Hospital No.	P-R + T.	T alone.	P-R alone.	No change.	Digitalis. Total.	Digitalis. Effective.	Time before death of: Change in electrocardiogram.	Time before death of: Last electrocardiogram.	Time in hospital.
						Without digitalis.				
						gm.	*gm.*			
1	2,305		+				0	7–0–0*	7– 0– 0	
2	1,918				0	0.4	0		1–12–39	
3	2,314				0	0.4	0		1– 1–20	1 curve
4	1,776				0		0		1– 0– 0	
5	2,157				0	0.4	0		0– 7–45	1 "
6	535				0		0		0– 5–10	Lead II only.
7	438				0		0		0–12–?0	" II " 1 curve. 2–5–25
						With digitalis.				
8	2,322†				0		0.7		0– 7–17	About 0–24–0 2 curves.
9	1,919‡				0		0.7		0– 7–50	" 2–0–0 1 curve.
10	2,308				0		1.3		0– 1– 0	3 curves.
11	2,205‡				0	0.3	0		0– 1– 0	About 0–24–0 1 curve.
12	1,899‡		+				About 0.4		0–12– 0	1–23–2 1 curve.
13	2,231‡		+			0.5	0.2		0– 8–50	About 1–4–55 1 curve.
14	2,339†		+			0.7	0.6	0–3–43	0– 3–43	1–4–13 2 curves.
15	2,355†		+			1.0	0.6	1–6–30		
16	2,268		+			0.9	0.8	0–4–50	0–4–50	About 2–2–20 3 curves.
17	1,963	+					1.2	0–8–0	0–8– 0	2 curves.
18	2,287	+				2.7	2.3	1–11–10		
19	2,295		+			1.6	0.9	1–21–8		
20	2,201		+			1.9	1.5	2–9–50		
21	2,230		+			2.2		3–0–0		
22	2,277	+	+				0	9–0–0		
				+		3.8	2.0	5–0–0		
23	2,208	+				1.3	1.1	6–0–0		
24	1,858	+	+				2.4	6–0–0		
				+		4.45	1.6	7–0–0		
25	2,247	+				3.3	1.1–1.5	12–0–0		

* The numbers 7–0–0, etc. refer to days, hours, and minutes, respectively.

† Repeated records not available.

‡ Single records not available.

year was 47.6 per cent. The number is small to serve as a basis for generalization, but it is clear that the administration of digitalis had nothing to do with the high mortality rate, for in each year approximately one-half the patients observed took the drug.

If the number of electrocardiographic changes was due to the high rate of mortality among the treated cases, the ground for this must be sought in alterations in the heart occurring during the agonal period or in the period 1 or 2 days before death. If the changes occur in the heart at that time, a high rate of mortality would naturally increase their number. It is necessary, therefore, to decide whether these changes occur at or about the time of death. We have accordingly analyzed the curves of all the patients (25) that died.

10 cases (Nos. 2 to 11) showed no changes (Table VII). 7 (Nos. 2 to 7, and 11) of these had taken no digitalis, while of the 3 (Nos. 8, 9, and 10) who had, 2 (Nos. 8 and 9) took insufficient amounts; that is, 9 (Nos. 2 to 9, and 11) behaved in the expected way. Of the 15 (Nos. 1, and 12 to 25) that showed changes, 1 (No. 1) had taken no digitalis, and 4 (Nos. 12 to 15) had taken insufficient amounts. The remaining 10 cases require no separate consideration; they had all taken enough digitalis to account for the result. The results, in summary, show that 19 of the 25 (76 per cent) patients either showed no changes when digitalis was not given, or given in insufficient amounts, or showed changes when sufficient amounts had been taken. The last curves examined in cases in which there were no changes in the curves were all taken from 1 to 36 hours before death; all but four were taken within 8 hours, and two within 1 hour. The fact that so many died without changes must be taken to mean that the proximity of death was not associated with the electrocardiographic changes on which we rely, even in those who had taken small amounts of the drug.

From a consideration of all the evidence we have been able to gather, we believe that the intoxication due to pneumonia exerts no influence against the action of digitalis. Digitalis produces the same effects in pneumonia, judged by the criteria we use, that it does in the absence of fever. We believe ourselves justified in assuming, therefore, that whatever actions the drug possesses, are exercised also during pneumonia. If the action is beneficial, advantage

may be expected from its use. That other observers have seen no results from giving it during severe pneumonia may depend on the fact that unjust criteria were employed for estimating its action.

SUMMARY.

We have shown in a series of 105 cases of pneumonia, 95 of which we have selected as available for statistical study, that digitalis given by mouth has an action on the heart. We have judged this action to be present because changes occurred in the auriculoventricular conduction time and in the form of the T wave of the electrocardiogram, just as they do in the non-febrile heart. This conclusion is strengthened by finding that the pulse rate in fibrillating and fluttering cases fell in the presence of fever, exactly as it does in non-febrile cases. The dose and the time required to produce these effects are given and are the same as in the non-febrile cases. When there was a difference in the amount necessary to produce one or the other of the changes, it was found that the T wave is more often and more readily affected than the conduction interval. We have shown that the intoxication due to pneumonia is probably not responsible for the changes found, both from a study of the statistics and because in the control cases reverse tendencies were often found (that is, decrease in conduction time and increase in the size of the T wave). We have shown that the method of selection in consequence of which we treated a large number of severe cases did not prejudice our results, because it could be demonstrated that the proximity of death, whether in control or treated cases, was not necessarily associated with the changes we are describing. We have also, by referring to the literature of the subject, brought evidence to show that heart muscle does not undergo those changes in pneumonia, as it does in other infectious diseases, which would lead one to expect changes in conduction found in other diseases. The changes in conduction which have been reported by others were almost entirely associated with the giving of digitalis.

CONCLUSIONS.

1. Digitalis acts during the febrile period of pneumonia.

2. It produces a beneficial, possibly a life-saving effect in cases of auricular irregularity (fibrillation and flutter).

3. Whatever beneficial action it has on the function of the normally beating non-febrile heart may be expected from its use in the febrile heart in pneumonia.

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EXPLANATION OF PLATE 12.

Fig. 1. Divisions of the ordinates equal 0.1 millivolt. Divisions of the abscissæ equal 0.04 second. In each set of these electrocardiograms, Leads I, II, and III are arranged from above downward.

A. These electrocardiograms were taken on January 24, 1915, the 3rd day after the onset of pneumonia. The temperature on this day ranged between 103° and 104.6°F. The P–R time was 0.15 second.

B. These curves were taken on January 26. The temperature ranged between 102.2° and 103.6° F. T_1, T_2, and T_3 are taller than in A. The P–R time was 0.16 second.

C. These curves were taken on February 3 during convalescence. T_1 and T_2 are taller than in either A or B; T_3 is less tall. The R–T_3 period is lengthened. The P–R time is 0.16 second. Apparently the T_1 and T_2 waves increased in size throughout the period of observation. The P–R time lengthened, and on February 28 was 0.21 second.

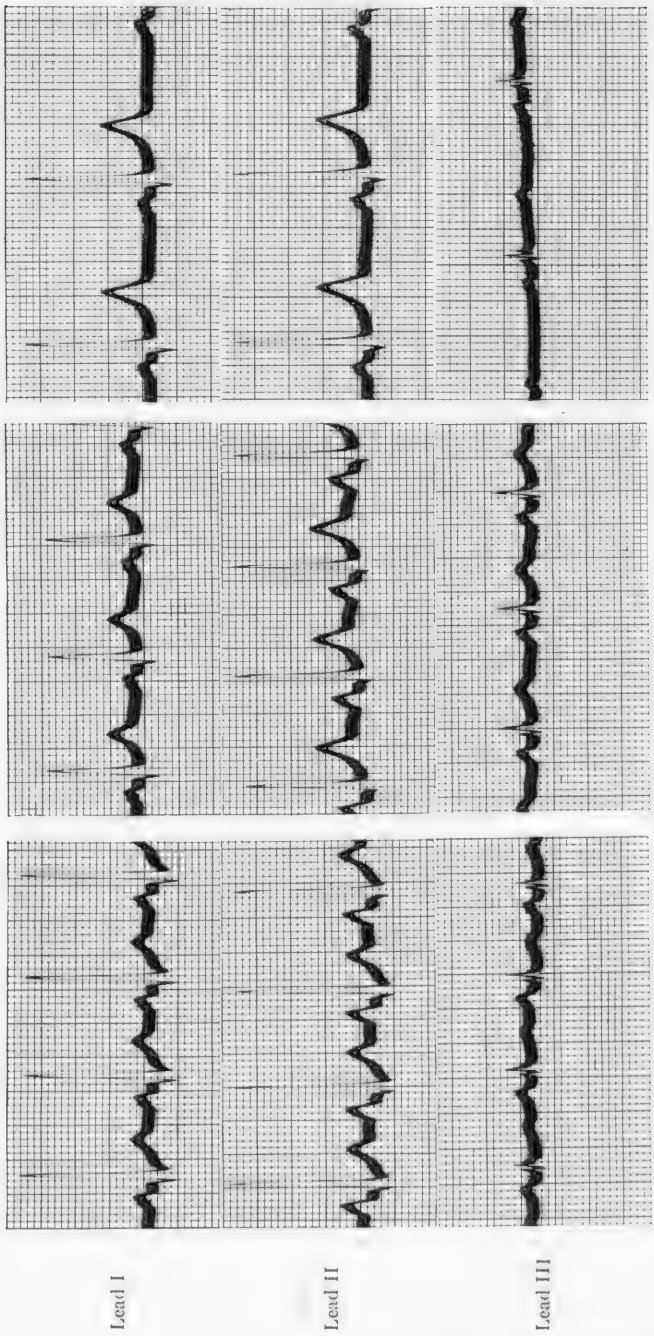

FIG. 1.

(Cohn and Jamieson: Action of Digitalis in Pneumonia.)

THE CONTROL OF STRYCHNINE CONVULSIONS BY INTRASPINAL INJECTIONS OF MAGNESIUM SULPHATE.

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(Received for publication, October 11, 1916.)

In cases of strychnine[1] poisoning, text-books on pharmacology and therapeutics (Kobert (1, 2), Tyrode (3), Cushny (4), and Dixon (5)), even of recent date, advocate evacuation and washing of the stomach by tube or by the usual emetics, accompanied by the administration of tannic acid or some other astringent, to remove the portion of the drug remaining in the stomach, and an attempt to control the convulsions by a depressant of the central nervous system, as urethane, chloral, bromides, ether, or chloroform. Kobert (2) advises, in addition, the use of small doses of curare, which must, however, be given with great care. Githens and Meltzer (6, 7) found that animals given a twice lethal dose of strychnine could invariably be saved by the use of intratracheal ether anesthesia plus the intravenous administration of considerable quantities of Ringer's solution. The success of their experiments apparently depended on the effect of Ringer's solution in hastening the excretion of the strychnine, the anesthesia merely controlling the convulsion until the drug was eliminated. Lethal doses were then rapidly excreted in the urine. The average duration of the anesthesia was 4½ hours.

The experiments of Githens and Meltzer were not known to us at the time of our study. Until now, little hope of recovery has been offered in cases where the convulsions have become general with opisthotonos, for respiratory paralysis soon follows.

Report of a Case of Strychnine Poisoning.

The present study was undertaken to confirm the results obtained in the following case.

Baby C., female, 1 year and 2 weeks of age; weight 22.5 pounds. Full term, normal delivery; breast fed for 1 month; healthy; no pre-

[1] U. S. census report for 1909 (see Wilbert, M. I., Poisons and Habit-Forming Drugs, *Public Health Rep.*, No. 330, 1916), gives strychnine poisoning as the cause of death 210 times, in 113 instances suicidal, and in 97 instances accidental.

vious illness. Admitted to the Massachusetts General Hospital, Accident Ward, at 5.30 p.m., November 25, 1915. 1 hour before admission the child had swallowed 12 to 14 pills which its father was taking for stomach trouble. The prescription showed these to contain $\frac{1}{60}$ of a grain of arsenic, $\frac{1}{60}$ of a grain of strychnine, and 1 grain of iron.

The mother gave the following history. 15 minutes previously the child stopped playing and had a slight general convulsion. It vomited once shortly after. The convulsive seizures were repeated in rapid sequence and the intensity increased. By the time the hospital was reached, the child was cyanotic and in an almost continuous convulsion, in which opisthotonos was frequent, with periods of total respiratory inhibition.

Ether was at once administered and the stomach tube passed. In the vomitus coming about the tube, and in the stomach washings three pills and several fragments of pills were recovered. Sodium bromide, 45 grains, was given by rectum but was at once expelled. The dose was repeated again but again the bowels moved. The same amount of the drug was administered by stomach tube but was later vomited. Urine was passed spontaneously. The color was better under ether. Twice the anesthesia was removed, but the child on both occasions had severe general convulsive seizures with opisthotonos and respiratory inhibition and was saved only by using artificial respiration to get it under the anesthetic.

On account of the clinical similarity of this case to a case of tetanus which had come under our observation, and the similar condition in the spinal cord, we determined to give magnesium sulphate intraspinally. Following the advice of Blake[2] (8, 9), we injected 0.9 cc. of a 25 per cent solution in the fourth lumbar space, keeping the head well elevated. The anesthetic was removed. The child at once recovered, seemed lively, and in no danger from respiratory paralysis. Apparently there was some loss of motor control in both legs, but without loss of the knee jerk reflex. Morphine, $\frac{1}{24}$ of a grain, was

[2] At that time we used the most easily available information as found in Keen's Surgery (Frazier, C. H., Tetanus, in Keen, W. W., Surgery, Philadelphia and London, 1907, i, 499). A more comprehensive article by Meltzer (19) since then advocates 0.5 cc. of a 25 per cent solution per 20 pounds in very young children.

given subcutaneously. The convulsions did not recur and the patient spent an undisturbed night, precautions being taken to keep the head elevated. Because of the slow excretion of strychnine in the urine, we thought that there might be a return of the condition the following day, but up to the time of discharge, December 5, 1915, there was no sign of even an increased motor irritability. A specimen of the urine was not obtained until December 4, at which time it contained no strychnine. Despite the large amount of arsenic also ingested, except for the vomiting and frequent evacuations of the bowels, the picture of acute arsenic poisoning was never presented, and later examination showed no evidence of chronic arsenic poisoning.

The action of strychnine (Kobert (1, 10), Tyrode (3), Cushny (4), Dixon (5), and Schmiedeberg (11)) consists in stimulation of the central nervous system, including the cord, medulla, and brain. Great reflex irritability results, with the characteristic rapid and active response to external stimuli. The action seems localized at some point between the entrance of the afferent fibers and the synapse about the motor cell. The easy response to stimuli from without seems best explained as the removing of some resistance "to the passage of impulses through some of the synapses in the spinal cord and thus extends the area on which an impulse acts, and also liberates it from the normal coördinating influences" (Cushny (4)). To control the action, then, it would seem reasonable to try to interpose an inhibiting influence at the point of the synapses.

In a series of papers Meltzer and Auer (12–18) discuss the toxicology, pharmacology, and therapeutic use of magnesium sulphate. They have demonstrated that the chief action of the drug is to inhibit and depress the central nervous system, simulating the action of cocaine when used locally. In spinal subdural injections its first action is to produce anesthesia, and later, with larger doses, paralysis. Thus it, too, may act in intraspinal injections, at much the same point as strychnine, though through a different mechanism and in an opposite direction.

In planning a series of experiments to test the efficacy of using magnesium sulphate to control strychnine convulsions, we found some confusion in the statistics recording the minimal lethal dose of strychnine. Githens and Meltzer (6) considered 0.0004 gm. per kilo of body weight fatal in dogs when administered intravenously. Hale[3] gave the same dosage from the results of his own experiments, also in dogs. Kobert (1) states that 0.0005 to 0.001 gm. per kilo is fatal for cats, dogs, sheep, and pigs.

[3] Personal communication.

EXPERIMENTAL.

We first planned a series of experiments to determine the minimal lethal dose (Tables I and II). Healthy medium sized cats were used. Strychnine was administered subcutaneously in the flank.

TABLE I.

Experiment No.	Weight of animal.	Strychnine.		Result.	Remarks.
		Amount per kg.	Amount injected.		
	gm.	*gm.*	*gm.*		
1	2,970	0.0002	0.0006	Well.	No convulsions; increased reflex irritability.
2	2,700	0.00035	0.0010*	"	No convulsions; increased reflex irritability.
3	3,900	0.0004	0.0016	Death.	General convulsive seizures began in 20 min. Death with inhibitory respiration in 1 hr.
4	2,200	0.0004	0.0009	"	General convulsive seizures began in 40 min. Death in 2 hrs.
5	3,560	0.0005	0.00175	"	General convulsive seizures began in 20 min. Death in 1½ hrs.
6	3,260	0.0005	0.00162	"	General convulsive seizures began in 20 min. Death in 30 min.
7	2,700	0.00065	0.0018	"	General convulsive seizures began in 24 min. Death in 50 min.

* In dealing with such small amounts of the drug the dosage given in some instances is approximately proportional to the animal's weight, not absolute.

TABLE II.

Experiment No.	Weight of animal.	Strychnine.		Result.	Remarks.
		Amount per kg.	Amount injected.		
	gm.	*gm.*	*gm.*		
8	1,405	0.0004	0.0006	Well.	Convulsion in 15 min. Ether to control it. Same procedure off and on for 1 hr. Then spastic, but no more convulsions.
9	2,970	0.0004	0.0012	Death.	Convulsions in 15 min. Controlled repeatedly by ether. Death in 1 hr. and 5 min.
10	3,150	0.0006	0.0018	"	Convulsions in 20 min. Controlled repeatedly by ether. Death in 1 hr.

These experiments convinced us that in cats the minimal lethal dose of strychnine was 0.0004 gm. per kilo. That we were able to control the convulsions entirely and prevent death by the use of ether (cone method) in Experiment 8, we attributed to the fact that the strychnine mixture as used in this experiment was a new one and not reliable in concentration. This preparation was discarded at once.

In the following experiments we went on the supposition that the same proportionate dose of the magnesium sulphate should be used intraspinally, as in children; *i.e.*, 1 cc. of a 25 per cent watery solution per 25 pounds.[4] Some difficulty was experienced, at first, in performing lumbar puncture on cats, but it was found that the space between the sacrum and fifth lumbar vertebra is sufficiently large and easily located because of the surrounding bony prominences.

In the first series of experiments (Table III) we injected strychnine subcutaneously, waited for a convulsive attack, anesthetized the animal, and injected magnesium sulphate subdurally. In the second series (Table IV), we first anesthetized the animal and then simultaneously injected strychnine hypodermically and magnesium sulphate intraspinally. We hoped by this method to show that we could abort the characteristic strychnine seizures. For anesthesia ether, by the cone method, was administered, except in one case in which chloroform was employed, and was used only long enough (often but 1 or 2 minutes), to enable us to complete our injections. The case, No. 12, in which we used chloroform died, probably because of the anesthetic, not the strychnine.

[4] The more recent advice of Meltzer (19) is 0.5 cc. per 20 pounds in very young children.

TABLE III.

Experiment No.	Weight of animal.	Strychnine.		Amount of magnesium sulphate.	Result.	Remarks.
		Amount per kg.	Amount injected.			
	gm.	*gm.*	*gm.*	*cc.*		
11	2,970	0.0004	0.0012	0.264	Well.	Convulsions in 20 min. After magnesium sulphate weak in hind legs, spastic in fore legs. Excitable but no more convulsions.
12	1,960	0.0004	0.0008	0.20	Death.	Severe convulsions in 30 min. Chloroform for 20 min. to control spasms. Then magnesium sulphate. Death before recovery from anesthetic.
13	3,060	0.0004	0.0012	0.375	"	For discussion see below. Magnesium sulphate cause of death.
14	3,900	0.0004	0.0016	0.352	Well.	Convulsions in 25 min. After magnesium sulphate paralyzed in hind legs. Quiet, no more convulsions.
15	3,450	0.0004	0.0014	0.308	"	Convulsions in 20 min. After magnesium sulphate weak in hind legs; slight spasm 1 hr. later; recovered from spasm.
16	3,260	0.0004	0.0014	0.286	"	Convulsions in 30 min. Weak in hind legs after magnesium sulphate. No more spasms.
17	3,235	0.0004	0.0013	0.286	"	Convulsions in 30 min. Weak in hind legs after magnesium sulphate. No more spasms.
18	2,970	0.0004	0.0012	0.264	"	Convulsions in 15 min. After magnesium sulphate no more spasms. Slight weakness in hind legs.
19	1,685	0.0004	0.0007	Total at 2 injections 0.308	"	Convulsions in 10 min. 15 min. after magnesium sulphate, repeated severe spasms in fore legs, none in hind legs. Dose of magnesium sulphate 0.154 cc. repeated. No more spasms. Playful with fore legs.
20	3,770	0.0004	0.0015	0.331	"	Convulsions in 25 min. No spasms after magnesium sulphate.

TABLE III—*Continued.*

Experiment No.	Weight of animal.	Strychnine.		Amount of magnesium sulphate.	Result.	Remarks.
		Amount per kg.	Amount injected.			
	gm.	*gm.*	*gm.*	*cc.*		
21	1,916	0.0004	0.0008	0.168	Well.	Convulsions in 30 min. No spasms after magnesium sulphate.
22	2,080	0.0004	0.0008	0.183	"	Convulsions in 50 min. No spasms after magnesium sulphate. Recovered. Died in 10 days of meningitis. The only case of infection.
23	3,235	0.0006	0.0019	0.280	Death.	Convulsions in 24 min. Flaccid in hind legs after magnesium sulphate. Repeated convulsions and death in 45 min.
24	2,880	0.0006	0.0017	0.260	"	Convulsions in 1 hr. and 25 min. After magnesium sulphate very excitable. Dead in cage next morning.

These experiments showed that we could save animals which had been given a lethal dose of strychnine, by the intraspinal injection of magnesium sulphate. The onset of the first strychnine convulsion though ushered in by a gradual increase in the irritability toward external stimuli, was usually very abrupt, and the spasms were so severe that frequently manual artificial respiration as a means of getting the animal under the effect of the anesthetic was undoubtedly the means of preventing death with the initial convulsion. The death in Experiment 12, was, we thought, due to the anesthetic. The death in Experiment 13 was due to magnesium sulphate poisoning. The effect of the drug as it diffused upwards in the spinal cord could be clearly seen. After recovery from the anesthetic the hind legs were noticed to be partially paralyzed, though frequent spasms seized the fore legs. Touching the hind legs called forth no reaction, while the slightest touch on the fur of a fore leg caused intense spasm of the upper half of the body. The animal was then laid on an inclined board with its head downwards. The effect of the magnesium sulphate moved steadily upwards, and areas on the abdomen and

chest which on previous light touch called forth spasm, no longer yielded the slightest response. Finally only the area from the neck upwards remained irritable. Soon afterwards the quality of the respiration became impaired and irregular. The head was then elevated and artificial respiration resorted to. Breathing was resumed and the animal lay in a semicomatose state for an hour longer, when respiration again failed and finally ceased.

The deaths in Experiments 23 and 24 were evidently caused by the excessive dose of strychnine. It does not seem improbable, however, that we could have saved the animals, by larger doses of magnesium sulphate, as we did in Experiment 19. In every case following the injection of the magnesium sulphate the same phenomena were observed: (1) flaccidity to almost total paralysis of the hind legs with failure of response to external stimuli; (2) continued increase of reflex irritability in the rest of the body, gradually diminishing and invariably progressing from the lower spinal region upwards. Few animals evinced any weakness in their hind legs 24 hours after the experiment.

In order to study further the power of magnesium sulphate in controlling the action of strychnine, we performed the following experiments, in which after anesthetization (necessary for the intraspinal injection), we injected strychnine and magnesium sulphate simultaneously (Table IV).

The results in these experiments seem to show that by using magnesium sulphate intraspinally early enough, the effects of the strychnine may be partially aborted. Possibly had we used larger doses of magnesium sulphate we should have been even more successful, but the death of the animal in Experiment 13 made us feel that we were near the high limits of the use of this drug.[5] The death of the animal in Experiment 27, though delayed, was probably due to strychnine, for we noticed no symptoms of the effects of the magnesium salts as late as 2 hours after the injection. Possibly in this case the full dose of the salt did not reach the subdural space. That the resultant convulsions in Experiments 28 and 29, despite the use of

[5] Meltzer (12) found that monkeys withstood 0.06 gm. per kilo intraspinally even when repeated two or three times at frequent intervals. Larger doses were fatal. In view of our experiments this seems a high dosage and suggests that strychnine may counteract the action of magnesium sulphate to some extent.

TABLE IV.

Experiment No.	Weight of animal.	Strychnine.		Amount of magnesium sulphate.	Result.	Remarks.
		Amount per kg.	Amount injected.			
	gm.	*gm.*	*gm.*	*cc.*		
25	3,150	0.0004	0.00126	0.268	Well.	In 30 min. weak in hind legs, but very irritable and susceptible to external stimuli. No convulsions.
26	2,690	0.0004	0.00107	0.240	"	Weak in hind legs and irritable. One general spasm in 35 min., then free from spasms.
27	2,500	0.0004	0.0010	0.220	Death.	In 35 min. one small convulsion. Weak in hind legs; irritable. Dead next morning.
28	2,880	0.0004	0.0012	0.260	Well.	In 20 min. severe spasm. Whiff of ether given. No more spasms.
29	3,170	0.0004	0.0012	0.276	"	In 30 min. severe spasm. Whiff of ether given. No more spasms; weak in hind legs.
30	2,455	0.0004	0.0010	0.220	"	Never any convulsions. Walked well. Slightly irritable.

magnesium sulphate, were so severe as to require a whiff of ether shows that the effect of the strychnine still dominated. Apparently there is a period about 20 minutes after subcutaneous injection when the toxic effect of strychnine is at its height. If at this time ether is given for a moment only, the magnesium salt will control further reactions.

SUMMARY.

We feel that the results obtained in the human case and in our animal experiments justify the supposition that magnesium sulphate may be of use in controlling cases of poisoning by strychnine. It is a method easily available not only in large hospitals but in private practice, and requires no elaborate technique. The amount of magnesium sulphate to be used should follow the advice of Meltzer (19) in tetanus cases: intraspinally 1 cc. of a 25 per cent solution to each 20 pounds of body weight in adults, and one-half the dose in young children. Should this not control the convulsions a small amount of ether may be used. In order to hasten the excretion of

strychnine, 200 to 300 cc. of salt solution should be given intravenously.[6] In the event of the return of the convulsions, the intraspinal dose may be repeated, always taking precautions to keep the head elevated. Meltzer's apparatus for intrapharyngeal insufflation and a 2.5 per cent solution of calcium chloride should always be on hand in case of respiratory failure following an overdose of the magnesium salts (Meltzer (19)).

We wish to express our indebtedness to Dr. S. J. Meltzer and Dr. J. Auer of The Rockefeller Institute for Medical Research for their interest and advice.

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[6] Githens and Meltzer (6) used Ringer's solution, but, of course, on account of the balancing action of calcium on magnesium salts (Meltzer (19)), this solution could not be used. Ringer's solution less the calcium chloride content, however, might prove more efficacious than plain salt solution in the elimination of strychnine.

STUDIES ON THE METABOLISM OF CELLS IN VITRO.

I. The Toxicity of α-Amino-Acids for Embryonic Chicken Cells.

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Plate 13.

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A synthetic medium suitable for the growth of tissue cells outside of the animal organism has not been discovered up to the present time. Since the preparation of such a medium would lead directly to a better understanding of cellular metabolism, this problem has stood forth as one of the most important of those presented by the tissue culture method.

From earlier observations evidence had already been obtained which showed that the ingredients essential for the building of new cells and the liberation of energy in the cultures comes directly from the tissue fragments. The growth observed is a manifestation of a simple transfer of materials from the more central portions of the fragment to the cells at the periphery, or, in other words, the preying of one cell in a more suitable environment upon its neighboring cells in an unsuitable one. This is true in cultures where simple isotonic salt solutions have been used as the medium. That it is also true in the case of the plasma culture can be readily shown by repeatedly changing the medium or transplanting the cells to drops of fresh medium. All activity ceases after a few transplants, or when the cells within the fragment have become exhausted.

One of us,[1] in a recent study, has shown that tissue cells are not highly organized elements. Their various manifestations of life are the result of reactions between themselves and a specifically

[1] Read before Section VIII, Subsection E, Second Pan American Scientific Congress and the American Cancer Society, Washington, January 6, 1916.

organized environment. The form of the manifested change is determined by the peculiar organization and composition of the environment. Growth associated with mitotic cell division is seen only in cells which have become passively placed at the interspace between certain insoluble substances and the medium. These substances are liberated from the cells when they are removed from their normal habitat to an oxygen-containing plasma or salt solution. The substances are almost transparent, their refraction is not very different from that of the original medium, and they accumulate at the surface of the medium to form a membrane. They are liberated in large amounts from a tissue fragment rich in cells.

A cell brought in contact with the surface of this transparent substance adheres to it and flattens over its surface. Such a cell grows and divides by mitosis when oxygen is present and the waste products remain below a certain concentration.

A single isolated cell when placed in a drop of fresh plasma does not grow and divide. It may show movement. Connective tissue cells often assume spindle and irregular stellate shapes. These simple activities lead, however, apparently to the exhaustion of these cells. They come to rest after a short period and show evidences of deterioration through vacuolization and failure to stain sharply.

All cells that grow in these cultures grow at the expense of their neighbors. In the culture the cells at the border of the fragment grow; they have come in contact with a layer of the transparent substances mentioned above, which was liberated by the large number of cells deteriorating in the center of the fragment. These cells within the fragment about which the border cells are growing actively soon become reduced to mere shadows. This deterioration goes on to such an extent that they no longer stain.

With the increase in the concentration of the waste products the activity of the cells in the outer medium not only becomes less and less, but there is also a decrease in the liberation of substances from the fragment. Eventually the whole system comes to a state of complete inactivity or equilibrium. Such a system remains without further change often for a long period of time, sometimes as long as 6 months, at incubator temperature and in the presence of an ample supply of oxygen. If the cells and the fragment at any time during

this period are removed to fresh medium, normal activity is again observed, provided the fragments and the cells had not been transplanted frequently at a previous time, or become exhausted of this substance, or of substances which lead to its formation.

If cells are placed in a medium where the mechanical conditions are such that these substances cannot diffuse out from the fragment over a surface, or the cells are prevented from coming in contact with this surface, growth followed by mitotic division does not take place, but other forms of change result. By merely changing the mechanical organization of the culture or changing the differential surface tension of the cell it has been found possible to change its form, structure, and the manifested activity associated with it. Heart muscle cells when placed in a medium where they may be passively carried against the flat surface of these substances lose their myofibrillar structure and grow and divide by mitosis. They resemble in every way growing sarcoma cells. The same cells may again be caused to grow to form a syncytium, their nuclei dividing by a mitosis. Again they may be caused to round off and resemble large mononuclears, or to differentiate into myofibrils and *vice versa*. The contracting heart muscle cell is a cell which has become stretched through a serum cavity between the surface formed by the substances diffusing from the fragment and an elastic band of fibrin. One end is adherent to the surface and the other to the end of the band of fibrin. The cell is elongated and spindle in shape. Its elongated shape is maintained by the stress of the fibrin. The surface of the cell, which is unattached to the fibrin or the surface of the substances coming from the fragment, is sharply defined. It has a high surface tension. The points of active change in such a cell are evidently the points of contact.

The cells of higher animals are, therefore, strikingly different from many unicellular organisms, such as the paramecium, etc., which contain within themselves all the necessary organization for their growth. Growth and the various manifestations of life of the animal cell are differential surface tension phenomena which are under the control of the environment. Their manifestations of life are manifestations of "tissue rather than cellular reaction."[1]

The growth of the cells in the culture depends upon the presence

of certain specific substances which are liberated by their neighbors and a specifically organized environment. The growth itself is evidently a highly specific reaction. It is a reaction which one might readily assume would continue as long as these specific substances are liberated from the fragment, the proper mechanical organization is maintained about the cells, ample supply of oxygen is present, and the waste products of the reaction are removed.

The questions that confronted us primarily in a further study of these cultures are: What is the nature of these substances? How are they formed in the body under normal conditions? Can any part of the body live an independent existence when isolated from the other parts? Is the organization of the culture such that it will be able to form these substances from simple food bases? The unicellular organism is capable of breaking down complex substances and building its various component parts from the products liberated. Are these systems which have been isolated, tissue cultures, also capable of an independent existence? Is the failure to demonstrate any actual increase of protoplasm in the plasma culture indicative that these organizations are not able to live an independent existence, to build these substances from the food bases, or is it indicative merely of the lack of the proper food bases in the plasma which has been used as a medium?

The fact that plasma contains carbohydrates, proteins, and fats made it seem plausible to assume that these systems are unable to utilize them directly as food substances. That in the ordinary plasma cultures these food bases are insufficient for the building of new cells can be shown definitely not only by the failure of the cells to continue to grow after several transplants, but further by actual measurements. The sum of the masses of new cells formed in each transplant is always less than the original mass. One could readily consider in a well regulated series that it represents the original mass minus the energy of transfer.

EXPERIMENTAL.

In the present series of experiments we tried to determine whether the addition of any substance to the plasma would prolong the growth of the cells. The addition of certain carbohydrates and fats did not

affect the growth to any degree. During the course of further experiments we had the opportunity to try certain hydrolytic products of the protein of egg yolk. Since the results of these experiments have a certain interest in themselves, we decided to report them separately at this time.

Method.

The tissues used for these experiments were heart muscle and pieces of the body wall of chick embryos and fetal chickens. The control medium for the experiments consisted of one part of fresh plasma containing a moderate amount of fat and one part of a 0.9 per cent sodium chloride solution. In the experiments the same proportions were used: one part of the isotonic solution of the substance to be tested was added to one part of the same plasma.

Fragments of heart muscle 1 mm. in diameter are placed in a layer of medium spread over the surface of a cover-glass. This layer has a thickness of 0.5 mm. A hollow ground slide is inverted over the drop, and the edge of the cover sealed to the slide with vaseline and paraffin. The cultures are placed in an incubator kept at a constant temperature of 39.4°C. They are examined under the microscope, which is kept in a chamber heated to the same temperature as that of the incubator.

After 3 days' incubation the growth of each is recorded and the fragment with the cells about it from each of the cultures is carefully transferred to a new drop of the medium, in which it has been previously grown. At the end of another 3 days the growth is again recorded and the fragments and cells again transplanted, and so on until no more activity is seen.

The growth in these cultures takes place as a rule along a single plane. A rough estimate of activity can be made by measuring the area covered by new cells about a fragment. There are always, however, a number of cultures in which the cells grow in several planes. On this account we have decided that a more accurate estimate can be given by the use of a relative term +, rather than by the direct numerical measurements. In the tables we have used this method of recording activity.

Plasma has been used in these experiments not only because it was of interest to test this medium more carefully, but also because

when it is used it is possible to obtain a constant and readily predicted cellular activity in the control cultures. This has not been found to be true for any of the simple liquid media. The proper mechanical conditions in the latter cultures are difficult of manipulation.

In the first series of experiments we prepared peptones from the yolk of eggs; that is, they were prepared from the specific food proteins of the embryonic chicken cells. These peptones were obtained by the use of the general method described by Abderhalden[2] and slightly modified by us as follows: 240 gm. of coagulated egg yolk are dried and pulverized, and treated with 1,000 gm. of 70 per cent (by weight) of pure sulphuric acid. The coagulated yolk does not dissolve as readily as one might wish, so the mixture is shaken vigorously for an hour. A thick scum forms on top, the rest of the material going into solution. This scum is removed. It proves to be fat. The brownish purple solution is next allowed to stand at room temperature for 3 days. It is frequently shaken during this time and then transferred into a 20 liter mixing jar, the latter being packed in a freezing mixture where it is cooled thoroughly. 10 liters of water are then added very slowly, while stirring, keeping the temperature below 20°C. After this, enough barium hydroxide in crystalline form is added to give the solution a neutral reaction towards litmus. This process requires great care. It is best to calculate the exact quantity of barium hydroxide needed, to add nearly this quantity, and then proceed gradually. The solution must be stirred constantly in order to keep the temperature below 20°C. at all times. The voluminous precipitate of barium sulphate is best separated from the digested solution by means of a centrifuge. This solution is next treated alternately with 0.1 N barium hydroxide and 0.1 N sulphuric acid until the addition of an excess of either to a filtered or centrifuged sample does not give a further precipitate of barium sulphate. Of course, an addition of barium hydroxide to a peptone solution will sometimes give a precipitate of barium peptonates, but these are soluble in dilute nitric acid, while barium hydroxide is not. Thus an absolutely neutral solution is prepared. This solution of over 10 liters in quantity is next concentrated under

[2] Abderhalden, E., Defensive Ferments of the Animal Organism, New York, 1914, 204.

reduced pressure until about 100 cc. of a syrupy, light yellow liquid results. This is treated with about 2 liters of hot methyl alcohol and filtered into 10 liters of chilled ethyl alcohol. A white precipitate results, and is quickly filtered off by the aid of a suction pump and dried in a desiccator. The color of the resultant powder is light yellow.

These peptones gave all the true peptone reactions. They were absolutely soluble in a saturated solution of ammonium sulphate and remained unchanged as regards color on boiling with a solution of sodium hydroxide to which a drop of lead acetate solution had been added. They were dialyzable through a dialysis tube, impervious to albumin, and the dialysate gave a typical blue ninhydrin reaction. Finally, they gave a reddish biuret reaction, and their microscopic appearance was similar to that of commercial silk peptone.

A large amount of this peptone, namely, 2.5 per cent, can be dissolved in 0.85 per cent sodium chloride solution, without making the solution hypertonic, and thereby killing the cells. This solution is mixed half and half with plasma. The growth, as is shown in Table I, Cultures 1, 2, 3, 4, and 5, continued regularly up to the sixth transplant. After this the various fragments became exhausted. The control cultures, 6, 7, 8, 9, and 10, behaved in exactly the same manner. From this we conclude that peptones do not in any way enter into this growth reaction. They did not prolong it, and no change of any kind was noted by their addition.

We next turned our attention to the study of α-amino-acids. The complete hydrolysis of egg yolk promised a yield of α-amino-acids in the same proportions as they actually occur in the protein molecule, the only α-amino-acid which does not give a yield on acid hydrolysis being tryptophane. This seemed to be the easiest and most direct way of procedure. Consequently a digestion mixture was prepared by dissolving dried egg yolk from which the fats had been extracted with ether by means of a Soxhlet apparatus, in a 70 per cent (by weight) solution of sulphuric acid. After the protein had gone into solution, enough water was added to make this a 25 per cent solution of sulphuric acid. The entire mixture was then heated in a flask with a reflux condenser until a biuret reaction was no longer obtainable. This acid solution was next neutralized with

TABLE I.

Tissue: Fragments of the heart muscle of a 12 day old chicken embryo.
Medium: Isotonic solutions of peptone, 2.5 per cent, in sodium chloride solution, 0.85 per cent, 1 part.
Plasma, 1 part.
Control Medium: Sodium chloride solution, 0.9 per cent, 1 part.
Plasma, 1 part.

Solution used.	Culture No.	Growth of transplants.						
		1st.	2nd.	3rd.	4th.	5th.	6th.	7th.
Peptone.	1	++++	++++	++	+	0	0	0
	2	+++	++++	+++	++	Few cells.	0	0
	3	+++	+++++	+++	+++	+	Few cells.	0
	4	++++	++++	++++	+++	++	Few cells.	0
	5	++++	+++++++	+++++++	+++++++	0	0	0
Control.	6	+++	+++++	—*	—	—	—	—
	7	+++++	+++++++	++++	++	0	0	0
	8	+++	++++	++	+++++++	++	Few cells.	0
	9	+++++	+++++++	+++++++	++++	0	0	0
	10	+++	++++	++	++	Few cells.	0	0

* In the tables the dashes indicate that the culture was lost through breaking of the glass slide, infection, or other causes.

barium hydroxide in exactly the same manner as stated above for the preparation of peptones. After this the solution was concentrated under reduced pressure at 40° C. until evaporation to dryness gave a 3 per cent residue, which we believe consists for the most part of α-amino-acids. Plasma was diluted with this mixture. To our surprise all the cells were dead in 48 hours, while the control cultures in plasma alone grew and kept on growing as usual when they were transplanted. We did not consider this peculiar result due to the possible toxicity of the α-amino-acids as such, but thought that traces of sulphuric acid or barium hydroxide caused the death of the cells. In order to make sure that this was the case, a part of the digestion mixture was further concentrated to one-fifth of its

volume, divided in two portions, and each portion tested with an excess of 0.1 N sulphuric acid and 0.1 N barium hydroxide respectively. No precipitation occurred.

Discarding the idea that a trace of sulphuric acid or barium hydroxide could be the cause for failure, we considered hypertonicity as a possibility. A simple way of testing whether a solution is isotonic or not towards chicken cells is by the use of chicken erythrocytes. The red blood corpuscles are nucleated and consequently have a bulge in the middle of the disc. This bulge is especially conspicuous when the corpuscle is seen on end. If a solution is hypotonic, the surrounding disc will swell and this bulge will become less marked. If, on the other hand, the solution is hypertonic, one sees characteristic shrivelling around the edge of the disc. It is, of course, understood that extremely hypotonic or hypertonic solutions will cause a dissolution of the corpuscles, a dissemination of the hemoglobin, and give a characteristic laked appearance.

Making up various dilutions of the digestion mixture, we transferred 1 cc. of the dilutions to suitable test-tubes and added 0.25 cc. of a 5 per cent suspension of washed chicken erythrocytes in 0.9 per cent sodium chloride solution. We then incubated it for 4 hours and compared it with the form of our control diluted with 0.9 per cent sodium chloride solution and treated in exactly the same manner. Using the dilution which showed neither shrivelling nor bulging, we prepared an isotonic digestion mixture.

Renewed experiments with the digestion mixture gave practically the same results as before. A repetition of the experiment with a new digestion again resulted in the death of the cultures. A portion of the last digestion mixture was treated with hydrochloric acid gas to the point of saturation and allowed to stand in the ice box for several days. We obtained a voluminous precipitate of glutamic acid hydrochloride, showing its typical crystalline form. This gave the final proof that α-amino-acids were present. Even now we did not attribute our results to a toxic influence of the acids in themselves but rather to the influence of the brown coloring matter in the solution, said to consist of caramelized carbohydrates, humin substances, etc. We therefore tried pure α-amino-acids.

We used ten of the acids in our first experiment; namely, glycocoll,

alanine, cystine, valine, leucine, phenylalanine, tyrosine, tryptophane, oxyproline, and asparagine.[3] Some of the substances were synthetic (Merck and Kahlbaum preparations), while others were obtained by the hydrolysis of proteins.[4]

To be sure of the substances which we used, the total nitrogen of each of the α-amino-acids was determined, their melting points were ascertained, and their crystalline forms observed. All these tests proved that we were dealing with chemical substances of the right molecular constitution containing no demonstrable impurities.

Hot saturated solutions of these acids were prepared. The solutions were cooled, and each solution was tested with chicken erythrocytes for isotonicity by the methods described above. The more insoluble substances, such as cystine, were primarily dissolved in isotonic salt solution, as we could hope to obtain isotonicity in no other way. All these solutions were diluted half and half with plasma and used as culture media.

Tables II, III, and IV give a survey of the experiments. In every case the addition of a solution of α-amino-acids to the plasma results in the ultimate death of the growing cells, while the controls keep on with their regular development. The discrepancy between the time required to kill the cultures described in Tables II and III, on the one hand, and in Table IV, on the other, the cultures of the latter series dying more quickly, is explained by the age of the embryo used. The cells of the younger embryo grow much faster and are less resistant to toxic influences than are those of an older embryo, as is known from numerous reported experiments.

[3] We used asparagine in spite of the fact that it has not been found as a constituent of hydrolyzed animal proteins. The effect as shown in the tables is exactly the same as in the case of the other α-amino-acids. Fig. 2 is a fair representative of the appearance of any one of the cultures in which α-amino-acids had been added to the medium. This culture was selected for photographing because it was prepared practically at the same time as the controls. The tissue that was used was most similar and had suffered practically the same exposure as that used for the controls.

[4] We wish to thank Dr. P. A. Levene, of The Rockefeller Institute for Medical Research, Dr. D. W. Wilson, of the Physiological Chemical Laboratory of Johns Hopkins University, and Dr. R. F. Ruttan, of McGill University, for supplying us with some of the acids more difficult to manufacture.

TABLE II.

Tissue: Fragments of the heart muscle of a 12 day old chicken embryo.
Medium: Isotonic solution of the α-amino-acid, 1 part.
Plasma, 1 part.
Control Medium: Sodium chloride solution, 0.9 per cent, 1 part.
Plasma, 1 part.

Solutions used.	Culture No.	Growth of transplants.	
		1st.	2nd.
Glycocoll, 35 per cent solution,* 1 part, and water, 19 parts.	1	+++	Few cells.
	2	+++	" "
	3	+++	" "
	4	+++	0
	5	+++	—
Alanine, 11 per cent solution, 1 part, and water, 13 parts.	6	++	+
	7	+++	Few cells.
	8	+++	+
	9	+++	0
	10	+++	Few cells.
Valine, 8 per cent, 1 part, and water, 4 parts.	11	+++	Few cells.
	12	++	0
	13	+++	Few cells.
	14	+++	0
	15	++	—
Leucine, 1.66 per cent, 1 part, and water, 2 parts.	16	+++++	0
	17	++++	0
	18	+++	0
	19	++++	0
	20	+++++	0
Cystine, 0.1 per cent solution, not diluted.	21	++	0
	22	+++	Few cells.
	23	++	+
	24	+++	+
	25	+++	0
Control.	26	+++	—
	27	++++	++++
	28	++++	—
	29	+++++	+++++
	30	++++	++++

* All percentages given are approximate.

TABLE III.

Tissue: Fragments of the heart muscle from an 11 day old chicken embryo.
Medium: Isotonic solution of the α-amino-acid, 1 part.
Plasma, 1 part.
Control: Sodium chloride solution, 0.9 per cent, 1 part.
Plasma, 1 part.

Solutions used.	Culture No.	Growth of transplants.			
		1st.	2nd.	3rd.	4th.
Valine, saturated solution, cold, 1 part, and water, 3 parts.	1	+	Few cells.	0	
	2	+	0	0	
	3	+	0	0	
	4	Few cells.	0	0	
Glycocoll (Merck), saturated solution, cold, 1 part, and water, 24 parts.	5	++	++	0	
	6	+++	+++	0	
	7	++	+++	0	
	8	+	+++*	0	
Asparagine, saturated solution, cold, 1 part, and water, 3 parts.	9	++	+*	0	
	10	+	+	0	
	11	+	+	0	
	12	+++	++	0	
Cystine, saturated solution, cold, 3 parts, and water, 2 parts.	13	++++++	+++	Few cells.	
	14	++++	++++	" "	+ Cells rounded off.*
	15	++++	++++	—	
	16	++++	—	—	
Controls.	33	+++++	++++++	+	+
	34	++	+++++	+	+
	35	++	++++++	+	+
	36	++++++	++++++++++	+	+

* All the pieces are contracting actively.

In Table V, we tried more dilute solutions, and, as this table indicates, the cells tend to resist this medium for a longer time. However, none of these cultures grew as long as the controls, which shows that even in very low dilutions the α-amino-acids are toxic. It is of interest in this series to note evidences of stimulation. This stimulation as the table indicates is not a stimulation of growth, but a stimulation of the contraction of the heart muscle fragments.

TABLE IV.

Tissue: Fragments of the body wall of a 5 day old chicken embryo.
Medium: Isotonic solution of the α-amino-acid, 1 part.
Plasma, 1 part.
Control Medium: Sodium chloride solution, 0.9 per cent, 1 part.
Plasma, 1 part.

Solutions used.	Culture No.	Growth after 48 hrs.	Growth after 72 hrs.
Glycocoll, 35 per cent solution, 1 part, and water, 19 parts.	1	+	The cells have all rounded off and show distortion.
	2	Few cells.	
	3	" "	
Alanine, 11 per cent solution, 1 part, and water, 13 parts.	4	+	The cells have all rounded off and show distortion.
	5	Few cells.	
	6	" "	
Cystine, 0.1 per cent solution, not diluted.	7	+	The cells have all rounded off and show distortion.
	8	+	
	9	+	
Valine, 8 per cent, 1 part, and water, 4 parts.	10	+	+++ The cells have all rounded off.
	11	++	+++
	12	++	+++
Leucine, 1.66 per cent, 1 part, and water, 2 parts.	13	++	++ The cells have all rounded off.
	14	++	++
	15	+++	+++
Phenylalanine, 2 per cent, slightly hypotonic, not diluted.	16	++	The cells have all rounded off and show distortion.
	17	—	
	18	+	
Tyrosine, 0.285 per cent in 0.85 per cent sodium chloride, 2 parts, and water, 1 part.	19	+	+ The cells have all rounded off and show distortion.
	20	++	+++
	21	++	++
Tryptophane, 1.66 per cent in 0.85 per cent sodium chloride, 1 part, and water, 2 parts.	22	++	The cells have all rounded off and show distortion.
	23	+++	
	24	++	
Oxyproline, 10 per cent, 1 part, and water, 2 parts.	25	+	The cells have all rounded off and show distortion.
	26	++	
	27	++	
Asparagine, 15 per cent, 1 part, and water, 1 part.	28	Few cells.	The cells have all rounded off and show distortion.
	29	" "	
	30	" "	
Control.	31	+++	++++++
	32	+++	++++
	33	+++	+++++

All the fragments contracted actively with the exception of the controls. This contraction continued to the complete exhaustion of the fragments.

TABLE V.

Tissue: Fragments of the heart muscle from an 11 day old chicken embryo.
Medium: Isotonic solution of the α-amino-acid, 1 part.
Sodium chloride solution, 0.9 per cent, 1 part.
Plasma, 2 parts.
Control: Sodium chloride solution, 0.9 per cent, 1 part.
Plasma, 1 part.

Solutions used.	Culture No.	Growth of transplants. 1st.	2nd.	3rd.	4th.
Valine, saturated solution, cold, 1 part, and water, 3 parts.	17	++++	—	—	—
	18	++++	+ Few cells.	0	0
	19	++	—	—	—
	20	+++	++++	+++*	+++* The cells have all rounded off.
Glycocoll (Merck), saturated solution, cold, 1 part, and water, 24 parts.	21	++++	+++++*	+++*	++++* The cells have all rounded off.
	22	+++++	+++++	+++*	0
	23	++++	++++	—	—
	24	—	—	—	—
Asparagine, saturated solution, cold, 1 part, and water, 3 parts.	25	++++	+++++*	—	—
	26	++++	+++++*	++++*	++++*
	27	—	—	—	—
	28	—	—	—	—
Cystine, saturated solution, cold, 3 parts, and water, 2 parts.	29	+++*	++++++*	+*	0
	30	+++++*	++++++	+++*	0
	31	++++*	++++++*	++++*	0
	32	+++++++*	++++++*	+*	0
Controls.	33	+++++	++++++	+++	+++++
	34	++	++++++	++++	+++++
	35	++	++++++	++++	++++
	36	+++++++	++++++++++	+++++++	+++

* All the pieces are contracting actively.

DISCUSSION AND SUMMARY.

Summing up these results, we found that all the ten α-amino-acids used inhibited the growth of the cells and finally killed the cultures. This inhibition is preceded by a short period of activity. The typical effect on the cells is shown in Figs. 1 and 2. The first (Fig. 1) is a control culture showing the usual growth of cells and their typical spindle shape form. The second (Fig. 2) is a culture in plasma plus asparagine,[3] showing the cells rounded off and beginning to undergo dissolution.

We do not wish to draw too extensive conclusions from these experiments, but we believe that the toxicity of α-amino-acids towards growing cells has been shown beyond a reasonable doubt; while we have found that compounds of higher molecular weight, namely, the peptones of egg yolk and proteins, are non-toxic. This toxicity depends upon the concentration and the time that the cells are exposed to their action. As these factors are reduced, the toxicity is decreased. In this respect, these substances are similar to all cell poisons.

Applying these results to the work done on the intravenous injection of digestion mixtures, we believe that we have found a reason for the death of the experimental animals when the hydrolyzed proteins were injected too rapidly. Buglia[5] found that large amounts of α-amino-acids could be injected into the circulation without causing deep-seated changes in the renal and intestinal functions, provided they were injected slowly enough; in fact, that enough of these mixtures could be injected in this way to cover the nitrogen consumption of the body. This injection, however, was always accompanied by an α-amino excretion through the urine and an increase of the peristalsis of the intestine, with resultant liquid stools. As is well known, a sudden great concentration of these substances in the blood of an animal causes death. These results agree with our findings.

Folin and Denis[6] demonstrated the fact that α-amino-acids probably pass into the circulation through the intestines. Van Slyke

[5] Buglia, G., *Z. Biol.*, 1912, xviii, 162.

[6] Folin, O., and Denis, W., *J. Biol. Chem.*, 1912, xii, 141.

and Meyer,[7] by means of Van Slyke's[8] nitrogen method, have practically proven this, and Abel, Rowntree, and Turner,[9] and Abderhalden[10] have lately succeeded in obtaining α-amino-acids in crystalline form from the blood. Van Slyke and Meyer[11] have shown that the tissues take up α-amino-acids to a certain point, but that after this the limit of saturation is reached. This is not so in the liver, which continually desaturates itself by metabolizing the α-amino-acids that it has absorbed, and consequently maintains indefinitely its power of removing them from the circulation, as long as they enter it no faster than the liver can metabolize them. Marshall and Rowntree[12] have shown that there is an increase of the α-amino-acid concentration in the blood after injuries to the liver, which have caused deep-seated anatomical changes. Our experiments prove that tissue cells in general are unable to live in the presence of any great concentration of these acids.

At the present time we do not feel able to give an explanation of the significance of this evident toxicity. However, the fact in itself seems to indicate that we should expect stimulation from a certain increase of the α-amino-acid concentration in the body, or the concentration of any one of the acids, while a greater increase would lead to marked disturbances of the metabolism.

EXPLANATION OF PLATE 13.

FIG. 1. The living cells which had grown from a fragment of the body wall of a 5 day old chick embryo into a medium consisting of 1 part of plasma and 1 part of isotonic salt solution, 0.9 per cent.

FIG. 2. The degenerated cells which had grown from a fragment of the body wall of the embryo used in the culture in Fig. 1 into a medium consisting of 1 part of plasma and 1 part of an isotonic solution of asparagine.

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[7] Van Slyke, D. D., and Meyer, G. M., *J. Biol. Chem.*, 1912, xii, 399.

[8] Van Slyke, D. D., *J. Biol. Chem.*, 1911, ix, 185; 1912, xii, 275.

[9] Abel, J. J., Rowntree, L. G., and Turner, B. B., *J. Pharm. and Exp. Therap.*, 1913–14, v, 275, 611.

[10] Abderhalden, *Z. physiol. Chem.*, 1913, lxxxviii, 478.

[11] Van Slyke and Meyer, *J. Biol. Chem.*, 1913–14, xvi, 213.

[12] Marshall, E. K., and Rowntree, L. G., *J. Exp. Med.*, 1915, xxii, 333.

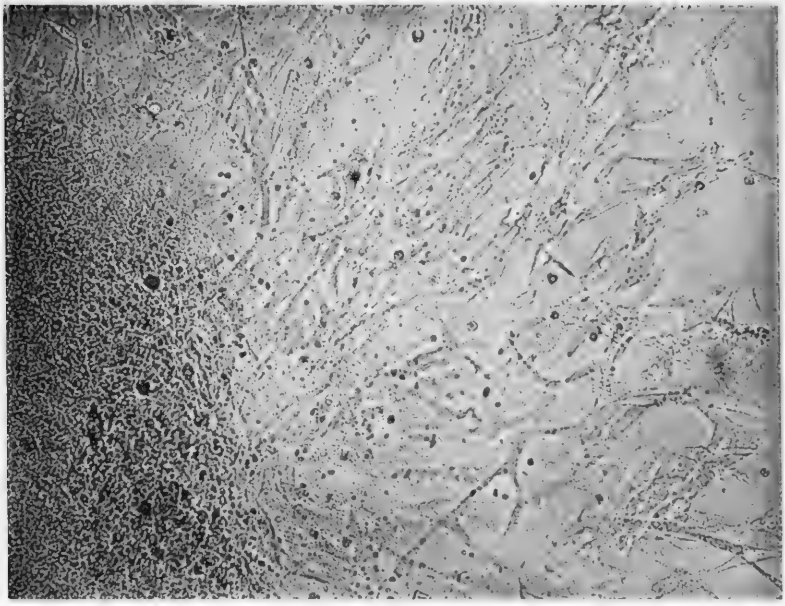

FIG. 1.

FIG. 2.

(Burrows and Neymann: Metabolism of Cells *in Vitro*. I.)

THE PRODUCTION OF ARTERIOSCLEROSIS AND GLOMERULONEPHRITIS IN THE RABBIT BY INTRAVENOUS INJECTIONS OF DIPHTHERIA TOXIN.

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Plates 14 to 19.

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Attention has frequently been called to the presence of vascular lesions in man, especially fatty streaks in the aorta, following infectious diseases, such as diphtheria, scarlet fever, and typhoid. This association has naturally led to an assumption by many of an etiological relation between infectious diseases and arteriosclerosis and to frequent attempts to produce vascular lesions in animals by the injection of bacteria or their toxins. Since the limits of this paper do not permit of an extensive review of this subject brief mention only will be made of the more important work and that which seems to bear especially on the experiments here reported. Critical reviews of the subject have been made by Klotz (1, 2,), Saltykow (3), Rickett (4), and Frothingham (5).

Gilbert and Lion (6) in 1889 traumatized the intima of the rabbit's aorta by passing a stylet down the carotid artery and afterwards injected cultures of *B. typhosus*. They thus obtained vegetative lesions. They also injected into two rabbits, without previous traumatization of the aorta, cultures of a bacillus obtained from a case of endocarditis. They produced plaques in the ascending portion and arch of the aorta, affecting mainly the media and consisting of fibrous tissue with some calcification.

Crocq (7) reported that traumatism of the aorta alone, by a method similar to that of Gilbert and Lion, produced no lesions; also that intravenous and subcutaneous injection of typhoid bacilli, colon bacilli, streptococci, and diphtheria bacilli, without previous traumatization, produced no lesions, but that when these bacteria were injected after previous injury to the aorta vegetations and plaques of endocarditis were produced. He therefore concludes that two factors are necessary, infection and a *locus minoris resistentiæ*.

Thérèse (8) and Pernice (9) injected *Staphylococcus aureus*, streptococcus, *B. typhosus*, *B. diphtheriæ*, etc., into rabbits and guinea pigs without previous injury to the aorta. They obtained microscopical lesions only which consisted of round cell infiltration and later fibrosis about the vasa vasorum, affecting mainly the adventitia, but in the more severe cases extending to the other coats.

Boinet and Romary (10) also tried trauma combined with infection. They used various bacteria, including diphtheria bacilli and also filtrates of cultures. Raised yellow patches of small extent were produced in the aorta which sometimes did not correspond to the traumatized areas. Microscopically these consisted of nodules of infiltration about the vasa vasorum with overlying plaques of endarteritis.

Saltykow (3) by repeated injections of staphylococcus into rabbits obtained small plaques of fibrous thickening and fatty degeneration of the intima of the aorta and some of its main branches, and sometimes cellular connective tissue nodules with calcification in the upper media.

Klotz (1) reports the production, by the injection of *B. typhosus* and streptococcus, of wart-like thickenings involving the intima and inner part of the media of the first part of the pulmonary artery and ascending limb of the aorta. He also mentions, without giving details of his experiments, the production of a vascular lesion in the rabbit with diphtheria toxin. On this subject he says: "The repeated inoculations of diphtheria toxin into rabbits gave surprising results. Here, instead of meeting with proliferative changes, such as the *B. typhosus* and streptococcus produce in the aorta, there were only lesions of a degenerative character. The degenerations were isolated to the first part of the aorta, and were identical with those produced in the adrenalin series. The thinning of the arterial wall, with calcification and aneurysmal dilatations, were all present, and the microscopical examination showed the lesions to be confined to the media. No proliferative or inflammatory changes were present in the intima, nor was there any change about the vasa vasorum."

Frothingham (11) remarks the fact that none of the vascular lesions which have been experimentally produced with bacteria or their toxins in animals are of so severe a type as those seen in man, and with the object of studying the effect of diphtheria toxin on the arteries he injected nine rabbits subcutaneously or intravenously. Some of these showed partial necrosis of tufts of the glomeruli with fibrin formation and in cases also there was necrosis of the walls of some of the larger vessels in the kidney with deposition of fibrin. Capillary lesions were also present in the adrenals. In no case were lesions of any of the larger arteries observed. Only two, however, of the nine rabbits survived over 3 days. One of these had five injections at intervals of 11 to 18 days and was killed at the end of about 10 weeks. The other had four injections at similar intervals and was killed in about 7 weeks. Two guinea pigs were also studied which survived an injection of toxin and antitoxin for about a month. No vascular lesions were present. The author concludes that though the glomeruli and smaller vessels in the rabbit's kidney are susceptible to diphtheria toxin, and also the capillaries

in the adrenal, "the vessels of these animals, however, are not on the whole sensitive enough to this toxin to make it seem hopeful to secure permanent lesions by the administration of sublethal doses."

The experiments here reported were undertaken in the hope of producing an arteriosclerosis of the larger vessels of the rabbit with diphtheria toxin. Lesions of the small vessels in certain organs have been described by various authors, as by Babes (12), Welch and Flexner (13, 14), and Frothingham (11) in the kidney, and by Mollard and Regaud (15) in the heart. The attempts of the earlier workers, in some cases successful, as quoted above, to produce vascular lesions by trauma and infection combined leave one in considerable doubt as to the relative importance of these two factors in the production of the lesion. The lesions obtained by Thérèse (8) and by Pernice (9) with bacteria or their toxins were slight and hardly comparable in character, severity, or extent with those in man which are suspected by some observers of being due to a preceding infection.

EXPERIMENTAL.

With the exception of the observation of Klotz (1), with which, however, the writer was unacquainted until these experiments were practically completed, the previous work seemed to afford little hope of producing arteriosclerosis with diphtheria toxin alone. An incentive to attempt to produce vascular lesions by the combined action of increased blood pressure and a toxin was furnished by a theoretical discussion of the etiology of arteriosclerosis by Aschoff (16). A number of rabbits were consequently injected with diphtheria toxin alone, a second series with diphtheria toxin and pituitrin, and a number, as controls, with pituitrin alone. The pituitrin was obtained from Parke, Davis and Company. It was given always intravenously. For the rabbits in Table II, it was usually mixed with the diluted diphtheria toxin, but occasionally was given undiluted a few minutes after the intravenous injection of the toxin; to the rabbits in Table III it was given undiluted. Pituitrin was selected because it was believed that of itself it would produce no vascular degeneration. This view was confirmed by the control rabbits Nos. 33, 34, and 35. Adrenalin was unsuitable on account of the medial degeneration produced by it in the aorta, as first pointed out by Josué (17), and since confirmed by many others.

All rabbits were injected with a single sample of diphtheria toxin obtained from the Cutter Laboratories, dilutions of which, freshly made each day before injection with sterile 0.85 per cent salt solu-

tion, were of such strength that the total amount of the injection was between 0.25 and 1 cc. The actual doses of undiluted toxin are given in the tables. The 0.4 per cent tricresol, added to the toxin by the manufacturers as a preservative, was disregarded under the assumption that the exceedingly small amount injected was without demonstrable morphological effect. All injections were made into the ear vein. The doses of toxin, of toxin and pituitrin, and of pituitrin alone are given in Tables I, II, and III. All the rabbits died from the effects of the treatment, except Nos. 33 and 34 which were killed at the expiration of the intervals shown in the tables.

All the animals were carefully autopsied with special attention to gross changes in the vessels. Frozen sections of various vessels and organs were stained with Sudan III and hematoxylin and with Van Gieson's stain and hematoxylin. Paraffin sections were stained with Van Gieson's stain and hematoxylin and with eosin and hematoxylin, and those of the vessels also with von Kossa's stain for calcium.

TABLE I.

Rabbits Injected with Diphtheria Toxin.

Rabbit No.	Weight.	Dosage of diphtheria toxin.	No. of doses.	Total dosage.	Duration of experiment.	Gross arteriosclerosis.	Nephritis.
	gm.	*cc.*		*cc.*	*days*		
1	2,340	0.02	1	0.02	1½	Absent.	Fatty degeneration.
2	2,520	0.015	2	0.03	6	"	Acute glomerulo.
3	2,470	0.006–0.007	4	0.025	4	"	Slight acute glomerulo.
4	2,985	0.004–0.005	4	0.017	6½	"	Moderate acute glomerulo.
5	2,240	0.003–0.004	3	0.013	9	"	Acute glomerulo.
6	2,245	0.002–0.003	9	0.019	28	Present.	Subacute glomerulo.
7	1,640	0.002	5	0.01	5	Absent.	Moderate acute glomerulo.
8	1,655	0.002	5	0.01	5	"	" " "
9	2,260	0.002	4	0.008	8	"	Acute glomerulo.
10	2,650	0.002	4	0.008	8	Present.	Beginning subacute glomerulo.
11	1,870	0.0015–0.0025	11	0.019	21	"	Moderate " "
12	1,670	0.0015	4	0.006	10	Absent.	Beginning " "
13	1,880	0.001–0.002	17	0.0235	24	"	Moderate " "
14	2,010	0.001–0.0015	12	0.0135	20	Present.	Subacute glomerulo.
15	1,900	0.0005–0.001	17	0.0125	26	Absent.	Slight subacute glomerulo.

TABLE II.

Rabbits Injected with Diphtheria Toxin and Pituitrin.

Rabbit No.	Weight.	Dosage of diphtheria toxin.	No. of doses of diphtheria toxin.	Total dosage of diphtheria toxin.	Pituitrin.	Duration of experiment.	Gross arteriosclerosis.	Nephritis.
	gm.	*cc.*		*cc.*		*days*		
16	2,010	0.03	1	0.03	2 doses of 0.4 cc.	1½	Absent.	Fatty degeneration.
17	3,200	0.02	1	0.02	2 " " 0.7 "	7	Present.	Acute glomerulo.
18	3,400	0.02	1	0.02	1 dose " 0.7 "	2½	Absent.	" "
19	3,450	0.02	1	0.02	1 " " 0.7 "	4	"	Slight acute glomerulo.
20	2,640	0.02	1	0.02	1 " " 0.7 "	2½	"	Slight glomerulo.
21	2,900	0.015	1	0.015	3 doses " 0.7 "	2½	"	Acute "
22	2,860	0.01	2	0.02	4 " " 0.7 "	3	"	" "
23	2,545	0.006–0.007	4	0.025	3 " " 0.7 "	5	"	" "
24	2,600	0.005	2	0.01	5 " " 0.7 "	5	"	Moderate acute glomerulo.
25	2,030	0.004–0.005	3	0.013	3 " " 0.7 "	5	"	Acute glomerulo.
26	3,360	0.003–0.004	4	0.013	4 " " 0.7 "	10	"	" "
27	2,645	0.002–0.003	4	0.009	4 " " 0.7 "	11	Present.	" "
28	1,465	0.002	5	0.01	5 " " 0.7 "	4	Absent.	" "
29	1,575	0.002	5	0.01	5 " " 0.7 "	9	"	" "
30	2,575	0.002	6	0.012	6 " " 0.75 "	14	Present.	Beginning subacute glomerulo.
31	2,240	0.0015–0.002	10	0.017	10 " " 0.75 "	20	"	Beginning subacute glomerulo.
32	1,615	0.0015	5	0.0075	5 " " 0.7 "	9	Absent.	Acute glomerulo.

TABLE III.

Rabbits Injected with Pituitrin.

Rabbit No.	Weight.	Pituitrin.	Duration of experiment.	Arteriosclerosis.	Nephritis.
	gm.		*days*		
33	2,260	4 doses of 0.5 cc.	4	None.	None.
34	3,110	23 doses of 0.5 to 1.0 cc.	23	"	"
35		2 doses of 0.8 cc.	3	"	"

In Tables I and II are also indicated the rabbits in which gross vascular lesions were found. A brief record of the vascular findings in these eight rabbits follows.

Rabbit 6.—The aorta is irregularly dilated especially in the thoracic portion. To the touch it is definitely stiffened. The large abdominal vessels, subclavians, and carotids are stiffened and the carotids show irregular dilatations. The aorta and carotids on opening show numerous transverse cracks and the lumen of these vessels is of varying diameter, an irregular thinning of the walls and shallow pouchings being present. Their walls are of the stiffness of parchment paper and many cracks are unavoidably produced by handling. The pulmonary artery is stiffened to its bifurcation. Sections of the vessels show a fatty degeneration of the smooth muscle of the media with crowding together of the elastic fibers into a compact layer. No calcification could be demonstrated.

Rabbit 10.—The aorta is irregularly dilated especially in the upper thoracic portion. To the touch it is stiffened throughout. The carotids are stiffened and of irregular diameter to the base of the skull. The subclavians, beginning of the large abdominal vessels, the iliacs, and about an inch of the proximal portion of the femorals are palpably stiffened. The aorta, on opening, has a scale-like appearance and shows shallow dilatations, and the abdominal aorta, carotids, subclavians, and iliacs show numerous transverse cracks. Sections of the thoracic aorta, abdominal aorta, and carotids show marked fatty degeneration of the smooth muscle of the media with crowding together of the elastic fibers into a compact layer. No calcification is present. There is considerable hemorrhage in the adventitia of the aorta.

Rabbit 11.—The thoracic portion of the aorta is slightly and irregularly dilated. The intima is of a dull gray color with fine closely set longitudinal striæ. No lesions are apparent in the abdominal aorta or any of the other large vessels. Sections of the arch show necrosis of the muscle in the media without calcification. There is hemorrhage into the adventitia.

Rabbit 14.—The arch of the aorta shows a moderate irregular dilatation. The intima of the thoracic portion is whiter than normally and shows a definite irregular thickening with longitudinal white striæ. The wall of the abdominal portion is stiff, and irregular areas of thinning are present. The carotids to the base of the skull, subclavians, iliacs, and the proximal inch of the brachials and femorals are slightly but definitely stiffened, of varying diameter, and on opening show numerous transverse cracks through the intima. Sections of the arch show an irregular marked cellular thickening of the intima without definite lesions in the media (Fig. 3). Sections of the abdominal aorta show a marked necrosis of the muscle of the media with crowding together of the elastic fibers, irregular thinning of the wall, and round cell infiltration in the adventitia. Sections of the carotid (Fig. 10) show necrosis of muscle, crowding of elastic fibers into a compact layer, and irregular thinning of the wall with small aneurysmal dilatations. No calcification could be demonstrated.

Rabbit 17.—An extreme diffuse lesion of the entire aorta from the aortic valves to the iliacs is present. The vessel is irregularly dilated, stiff, and definitely calcified throughout. A similar lesion involves the innominate, carotids, subclavians, large abdominal vessels, iliacs, and the brachials and femorals for a considerable distance. On palpation these vessels crackle under the finger like thin parchment paper and cracks are easily produced by bending. The pulmonary artery shows no gross lesion. Sections of the thoracic and abdominal aorta show a marked diffuse fatty degeneration and calcification of the media involving usually the inner third or half. The intima, and in places a thin layer of media overlying the zone of calcification, is necrotic and without calcium (Fig. 5). There is some hemorrhage into the adventitia. Sections of the carotids, iliacs, femorals (Fig. 11), etc., also show extensive necrosis and calcification of the media, generally diffusely involving the inner third, but in places extending nearly to the adventitia. The renal artery is similarly calcified to the kidneys. Small plates of calcification in the inner media of a large branch of the renal artery in the pelvis of the kidney are shown in Fig. 13.

Rabbit 27.—The lesions are of the same character, distribution, and severity as in No. 17, except that in this rabbit two small calcified plates, each about 3 mm. in diameter, are present in the pulmonary artery near the base. Sections of the arch (Fig. 4) and thoracic aorta show everywhere a wide zone of necrosis and marked calcification in the media. This calcified layer is of fairly uniform width in the arch but runs a wavy course, varying considerably in its distance below the intima. The elastic fibers themselves appear to be calcified. The intima and portion of the media overlying show considerable necrosis. In the abdominal aorta this overlying tissue, as well as the calcified material itself, in places, has sloughed off leaving deep ulcers (Figs. 7 and 8) which sometimes extend nearly to the adventitia, the latter showing hemorrhage and round cell infiltration. Small aneurysmal dilatations are numerous (Fig. 8). The carotids (Fig. 12) and iliacs also show extensive necrosis and calcification of the media.

Rabbit 30.—(Figs. 1 and 2.) The lesions are practically the same as in Nos. 17 and 27, but calcification of the iliacs is not so pronounced and is less extensive, and calcification of the brachials and femorals is not evident in the gross. The pulmonary artery shows a distinct fusiform dilatation (Fig. 1), the wall of which contains calcareous plates. The carotids are markedly stiffened to the base of the skull but no lesions of the vessels at the base of the brain, or in its substance, were evident in the gross or microscopically. Sections of the thoracic and abdominal aorta show similar widespread necrosis and calcification of the media with extensive and deep ulcers (Figs. 6 and 9), and hemorrhage and round cell infiltration in the adventitia. Sections of the smaller arteries also show extensive and practically uniform calcification of the media.

Rabbit 31.—The aorta on external inspection appears normal. The intima is whiter than normally and shows a slight but definite irregularly distributed thickening, more pronounced in the thoracic portion. The lower abdominal aorta for about 2 cm. above the iliacs is definitely stiffened and shows transverse cracks.

The pulmonary artery and branches of the aorta appear normal. Sections of the thoracic aorta show an irregular cellular thickening of the intima with no definite lesions in the media. Sections of the lower abdominal aorta show fatty degeneration of the muscular and elastic tissue of the media.

DISCUSSION.

The question at once arises whether the striking vascular lesion found in these rabbits is due to the treatment or preexisted as a spontaneous arteriosclerosis. That the latter occurs there can be no doubt, but in the author's experience it has been rare, only one case having been observed in a large number of rabbits whose vessels were carefully examined, and in this animal the lesion consisted of a single 2 mm. plaque of intimal thickening in the arch. Miles (18), however, reports the occurrence of spontaneous arteriosclerosis in seventeen out of forty-nine rabbits. In all these, however, the lesions were small and usually situated at the point of exit of a large vessel from the aorta. Whatever may be true as to the incidence of arteriosclerosis in rabbits no case has been reported, in so far as the author can discover, in which the lesions at all approximate in severity or extent those here reported. Even the vascular degeneration produced in rabbits by the administration of adrenalin chloride, first described by Josué (17), and since confirmed by others, but believed by some to be an independent and preexisting lesion, involves only the aorta and pulmonary artery in the experience of the writer, and it is believed that this accords with the observations of others; and even in the aorta the lesion is less severe and diffuse than that observed in these animals. The vascular lesion in these rabbits is so pronounced, in the peripheral vessels affected as well as in the aorta, that it is quite evident, after exposure of the vessel, on external inspection and even more so on palpation.

Several inconsistencies in the results obtained are, however, at once apparent on examination of the tables, as the presence of a pronounced arteriosclerosis in No. 14 and its entire absence in No. 13; also in Nos. 9 and 10. In order to produce the lesion it seems to be necessary to have a large amount of unbound toxin circulating in the blood and to produce this large doses must be injected before immunity is established. That a vascular lesion might not ultimately

result from frequently repeated small doses is not proven by these experiments, but as far as they go they tend to show that this is the case. The lack of results reported by Frothingham (11) on two rabbits as previously quoted, also gives strength to this view. The impression has also been gained that it is easier to produce the lesion in large rabbits. The differences in results are not, however, entirely explainable on the basis of dose, time, or weight of the animal, nor do individual differences in susceptibility to the toxin seem an adequate explanation.

Briefly summarized, the lesions observed consist of a fatty degeneration and necrosis of the smooth muscle of the media with subsequent crowding together of the elastic fibers into a relatively compact layer. There is also a degeneration of the elastic tissue in the media and the degenerated tissue becomes extensively calcified. In severe cases there is also a necrosis of the overlying intima and deep ulcers are formed. In certain rabbits there is a definite cellular hyperplasia of the intima which appears to be independent of medial degeneration. This will be discussed later. In none of the animals in which extensive necrosis and calcification were observed was there any inflammatory reaction in the media or hyperplasia of the overlying intima. In these cases, however, the overlying tissue was largely necrotic and sufficient time had perhaps not elapsed for reparative or compensatory processes to take place. It is quite possible that regeneration and compensatory intimal hyperplasia might ultimately occur over these medial lesions if the animals could be kept alive. The cracks mentioned as occurring both in the aorta and peripheral vessels are to a considerable extent unavoidably produced post mortem by handling the vessels. That some of these fractures, however, which extend deep into the media, existed during life is proved by the fact that in several cases small dissecting aneurysms were found in the aorta which had manifestly been produced by the entrance of blood through the rupture in the intima and inner media into the necrotic zone in the deeper media. In one case a definitely antemortem clot had formed in one of the cracks and protruded as a small thrombus into the lumen of the vessel.

It is to be noted that four of the fifteen rabbits which received diphtheria toxin alone (Table I) showed gross vascular lesions as

well as four of the seventeen rabbits which received toxin and pituitrin (Table II). It will also be noted that no calcification was demonstrated in any of the animals receiving toxin alone, while three of the animals receiving pituitrin with the toxin showed widely distributed and marked calcification. Between the two series, however, there was little difference in the gross characteristics or distribution of the lesions. The vessels in Nos. 6, 10, and 14 were almost as stiff and brittle as those in Nos. 17, 27, and 30, yet in the first three no calcification could be demonstrated either with hematoxylin or the von Kossa stain, while in the last three calcification was marked and widely distributed. Definitely palpable stiffening and a brittleness of such a degree that the vessel cannot be opened without producing many cracks extending through the intima and deep into the media are present before calcification takes place, due apparently to a degeneration of the elastic tissue which occurs in these severe cases along with the muscular degeneration. The severity of the lesion in these rabbits is shown in Fig. 10. Why calcification should occur in the fatty degenerated medial tissue of the rabbits which have received pituitrin, and not in the rabbits which have not, is difficult to explain. The two series differ only in the presence or absence of calcification, and it is felt that no emphasis should be laid on the importance of pituitrin in the production of this change. Possibly in a larger series this difference would not be so apparent. In fact, as already noted, Klotz (1) has obtained small plaques of medial calcification, limited, however, to the first part of the aorta, with diphtheria toxin alone. No case with small focal lesions was found in this series, the gross lesions being either diffuse and pronounced or entirely lacking.

The resemblance of these vascular lesions to the adrenalin type of arteriosclerosis has already been mentioned, and Klotz has called attention to the similarity of the latter lesion to the Moncheberg type of arteriosclerosis occurring in the peripheral vessels of man. In this connection it would seem appropriate to call attention to the following facts: that the lesions which have been experimentally produced with staphylococcus (Saltykow (3)), typhoid, and streptococcus (Klotz (1)) have been of the proliferative type affecting primarily and mainly the intima of the aorta and that lesions of the

peripheral vessels, except for focal lesions with staphylococcus, have not resulted; that the lesions obtained with diphtheria toxin have been degenerative and medial, closely resembling the Moncheberg type, but unlike this type in man, have been confined to the beginning of the aorta (Klotz (1)), or have involved the entire aorta as well as to a considerable extent the peripheral vessels (present experiments); and that, on the other hand, the lesions which have been mainly associated, from clinical and postmortem observation, with infectious diseases in man have been the proliferative intimal type affecting the aorta, and an acute degeneration of the smaller arterioles in some of the organs, such as the kidneys. From such results as these here reported one is tempted to argue for an infectious or toxic origin for this peripheral type of arteriosclerosis in man, characterized by medial degeneration and calcification; and, from the results reported with staphylococcus, streptococcus, and typhoid bacillus, for a similar origin for the intimal plaques of proliferation and fatty degeneration in the aorta. Moreover, the fact that up to the present the injection of different varieties of bacteria or their toxins into rabbits has resulted in different types of arteriosclerosis at once suggests a specific etiology for these types in man. One seems, however, hardly justified in so applying these results, especially to the extent of the last conclusion. The intima of the rabbit's aorta is very thin, consisting practically of a single layer of endothelial cells lying on the internal elastic lamina of the media, and the facts above recorded may possibly be largely accounted for by anatomical structure of the vessels and strength, rather than specificity of toxin. A weaker toxin might produce proliferative rather than degenerative lesions; a stronger toxin might produce degeneration of more highly differentiated cells, as smooth muscle, before endothelial cells or fibroblasts. Since the vessel walls in these rabbits are evidently affected by toxin from the lumen rather than through the vasa vasorum we obtain in these animals, with a thin aortic intima, a medial degeneration of the aorta as well as of the peripheral vessels. If these theoretical explanations are correct we might expect to obtain with a proper strength of toxin in an animal with a thick aortic intima a proliferative intimal lesion here and a degenerative medial lesion in the smaller vessels; and in the rabbit one might hope to find a smaller dose of toxin which would produce a

proliferative intimal lesion in the aorta and a medial degeneration in the smaller vessels, which are without the dense elastic laminæ of the aorta and probably more permeable. In one rabbit, No. 14, which received a relatively small dose of toxin, we find this condition present—intimal hyperplasia in the aorta (Fig. 3) and medial degeneration in the smaller vessels (Fig. 10)—thus giving a certain amount of experimental evidence for the idea that the difference in the type of lesion experimentally obtained with staphylococcus, streptococcus, and typhoid bacillus on the one hand, and diphtheria toxin on the other, are not due to specific action on a certain type of cell but to strength of toxin and anatomic structure.

Of possible interest in connection with these experiments is the work done by various authors on the effect of diphtheria toxin on adrenal function. Langlois (19), Luksch (20, 21), and Hannes (22) claimed that diphtheria toxin decreased adrenalin secretion, and Ehrmann (23) claimed that it increased it. Bonnamour and Thévenot (24) injected rabbits subcutaneously with diphtheria toxin and at the same time intravenously with adrenalin. They believe that the aortic degeneration resulting from the combined action of diphtheria toxin and adrenalin is more severe than from adrenalin alone, and that therefore the adrenalin secretion is increased. They injected no animals with diphtheria toxin alone. Tscheboksaroff (25) believes that the previous disagreement is explained by his results on dogs. He found that for 10 to 15 hours after the injection of diphtheria toxin the adrenalin secretion was increased, that in a second stage occurring 24 to 27 hours after injection the adrenalin secretion was normal, and in a third stage, 48 to 96 hours after injection, adrenalin disappeared from the blood. Abramow (26) from work on rabbits, guinea pigs, and immunized horses, concluded that under the influence of large doses of diphtheria toxin the adrenalin secretion ceased, with minimum lethal doses it decreased, and with sublethal doses and in immunization it increased. Some of these results, as well as the morphological resemblance between this and the adrenalin type of arteriosclerosis, suggest the possibility of a relationship between the two. The fact that the adrenals of these rabbits are enlarged seems no evidence of an increase in secretory function. The adrenals are also enlarged in rabbits injected with other bacteria, as in a series injected over long periods with colon bacilli by the author (27), in none of which were any lesions of the large vessels present. The enlargement of the adrenals is probably due to the storage in the cortex of fat, largely anisotropic, from the fatty degeneration produced by the toxin. If there is an increase in secretory function it is independent of this enlargement. It seems probable that the vascular lesion reported is due to the direct action of the diphtheria toxin on the vessel walls rather than through the medium of adrenalin. The latter possibility cannot, however, be absolutely dismissed.

It is not the purpose of this paper to recount the acute changes produced in various organs by diphtheria toxin, which have been previously carefully studied and described by others. It is desired, however, to mention briefly the changes in certain organs. The spleens of the arteriosclerotic rabbits which withstood the treatment longest are in marked contrast, as to size, with those of the rabbits which lived only a few days and particularly with those of rabbits, previously reported (27), which have been injected with colon bacilli. In older arteriosclerotic rabbits the organ is shrunken, being one-third to one-half the normal size; microscopically the Malpighian bodies are atrophied and there is a diffuse increase of connective tissue throughout the reticulum of the pulp. In the rabbits injected with colon bacilli the spleen is much enlarged; microscopically there is atrophy of the Malpighian bodies with a marked deposition of amyloid. In the rabbits injected with diphtheria toxin the arterioles in the spleen show no marked lesions, but that their endothelium is affected is evidenced by the fact that the lumen sometimes contains large numbers of flat, desquamated, endothelial cells.

Babes (12), experimenting on rabbits with diphtheria toxin, describes swelling, proliferation, and desquamation of the epithelium of the kidney and also of the endothelial cells of the blood vessels with the formation of hyaline masses in their lumina. Welch and Flexner (13), working on guinea pigs, rabbits, and kittens with diphtheria toxin, describe hyaline alteration of the glomerular capillaries and smaller arteries, especially in kittens. The hyaline completely filled the lumen of some capillaries. There was also fragmentation of nuclei in the glomeruli and tubular epithelium on a small scale. Frothingham (11) has described necroses of the walls of the larger vessels of the kidneys in rabbits, with fibrin deposits in the lumen and hemorrhage into the surrounding tissue. The glomeruli in cases showed partial necrosis of the capillaries of the tufts with fibrin formation in them.

The results in these rabbits confirm in the main the previous observations. In the gross the kidneys were of normal size or somewhat enlarged, they were frequently deep red, but sometimes pale and opaque. The minute dark red spots, characteristic of glomerular hemorrhages, were frequent. In a few of the more typical cases these glomerular hemorrhages, and also small opaque white spots were thickly scattered over the surface and on section were seen in the cortex. No differences were noted, either in the gross or micro-

scopically, between the kidneys of the rabbits which received toxin only and those of the rabbits which received pituitrin with the toxin unless it was that the hemorrhages were somewhat more numerous in the gross in the latter series. Even in this regard, however, the difference was not striking. Microscopically both vascular and glomerular lesions were present in nearly all the rabbits, as shown in Tables I and II, and in most cases were frequent and well marked. The vascular lesions consisted of swelling and desquamation of the endothelial cells of the arterioles and small veins with numerous lateral thromboses in these vessels. The glomeruli show necroses of the capillaries with hemorrhages into the tufts and capsular spaces and the formation of fibrinous and hyaline masses (Fig. 14). Casts and red blood corpuscles are numerous in the tubules. There is considerable pyknosis and fragmentation of the nuclei, and in some cases a swelling and apparently early cellular proliferation in the tuft. Some of the glomeruli show rather marked collections of polymorphonuclear leukocytes in and about the lesions in the tufts (Fig. 15). It seems appropriate to call particular attention to the occurrence of these focal glomerular lesions with cellular infiltration in the kidney, produced by a soluble toxin circulating in the blood, as did Welch and Flexner (14) in the case of the focal lesions in the liver. A few of the older cases show an early diffuse interstitial proliferation of connective tissue, apparently quite different from the focal scars occurring as a spontaneous lesion in rabbits. The Sudan stain shows that fatty degeneration was always present, but varying much in degree and extent. In the less severe cases the convoluted tubules only are affected; in the more severe cases the tubular epithelium is affected throughout. Moderate fatty degeneration in the tufts is frequent.

Though these lesions do not differ essentially from those previously reported, it would seem that the similarity of the nephritis to acute glomerulonephritis in man has not been sufficiently emphasized. Ophüls (28) has called attention to the importance of vascular changes in glomerulonephritis in man, and, in these rabbits, the primary lesion and essential factor in the production of the glomerular change appear to be a similar vascular damage and the result is a nephritis closely resembling the acute and subacute glomerular types in man.

It is unfortunate that it has thus far been impossible to keep a rabbit alive for more than 28 days after the injection of the large dose of toxin which seems essential for the production of the vascular lesions here reported, since a study of the subsequent morphological changes both in the vessels and in the kidneys, as well as of the functional changes resulting, would be of much interest.

CONCLUSIONS.

There may be produced in rabbits by the intravenous injection of large doses of diphtheria toxin a vascular degeneration involving the entire aorta, the carotids to the base of the skull, the subclavians, and iliacs, and, for a varying distance distally, the brachials, femorals, and large abdominal vessels. The first part of the pulmonary artery is sometimes affected. The lesion is practically diffuse throughout the aorta and vessels mentioned, consisting of a fatty degeneration and necrosis of the smooth muscle in a wide zone of the media and a crowding together of the elastic fibers in the region affected, resulting in an irregular thinning of the vessel walls and many small aneurysmal pouchings. In rabbits which received pituitrin with the diphtheria toxin extensive calcification occurred throughout this degenerated zone, both in the aorta and other large vessels. It is believed, however, that the pituitrin is not essential to the calcification and that if it is of any importance it is because an extreme fatty degeneration is produced more quickly in the media of the vessels when it is administered simultaneously with the toxin.

Diphtheria toxin, given in large doses intravenously, produces in the kidneys of the rabbit a pronounced vascular and parenchymatous degeneration. The former consists of a swelling and desquamation of the endothelial cells of the arterioles and small veins with the formation of fibrinous thrombi, a necrosis and thrombosis of the capillaries of the tufts with hemorrhage and the formation of fibrinous and hyaline masses, and in some of the affected glomeruli considerable collections of polymorphonuclear leukocytes.

In conclusion I wish to acknowledge my indebtedness to Dr. Ophüls for his interest and advice throughout the experiments, and particularly for his examination of the kidneys.

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EXPLANATION OF PLATES.

PLATE 14.

FIG. 1. Rabbit 30. The aorta and its branches. All the vessels shown were diffusely calcified with the exception of the abdominal branches of the aorta and the iliacs. In these calcification was only slightly apparent in the gross. The irregular dilatation of the upper portion of the aorta is shown ending sharply at about the level of the right renal artery. A shallow but well marked dilatation is seen on the convex surface of the descending portion of the arch just below the left subclavian artery; also a fusiform dilatation of the pulmonary artery lying just above the left auricle. The irregular thinning of the walls is apparent, particularly in the abdominal aorta and the carotids. The transverse cracks are apparent even in this external view, seen best in the abdominal aorta and right carotid.

FIG. 2. Rabbit 30. The same as Fig. 1, but with some of the vessels opened. Note the irregular dilatation of the aorta ending sharply at about the level of the right renal artery; also the circumscribed dilatation of the aorta just below the left subclavian, a similar dilatation on the posterior wall of the proximal portion of the right carotid, and the fusiform dilatation of the pulmonary artery (not opened) lying just above the left auricle. The stiffness of the vessels is evidenced by the crumpled appearance, best seen throughout the thoracic aorta, and their brittleness by the numerous transverse cracks, best shown in the photograph throughout the aorta, but also present in the other vessels.

PLATE 15.

FIG. 3. Rabbit 14. Arch of the aorta. A marked cellular thickening of the intima is seen, without apparent lesions in the media. Microphotograph. Hematoxylin and Van Gieson's stain. Bausch and Lomb obj. $\frac{2}{3}$, oc. 1.

FIG. 4. Rabbit 27. Arch of the aorta. A wavy zone of marked calcification in the media is present, of fairly uniform width but varying in depth below the intima. There is considerable necrosis with lack of nuclear staining in the overlying intima and media. Microphotograph. Hematoxylin and Van Gieson's stain. Bausch and Lomb obj. $\frac{2}{3}$, oc. 1.

FIG. 5. Rabbit 17. Thoracic aorta. There is a zone of marked calcification in the upper media with necrosis of the intima and overlying media. On the left, calcified spicules have broken through and project into the lumen. Microphotograph. Hematoxylin and Van Gieson's stain. Bausch and Lomb obj. $\frac{2}{3}$, oc. 1.

PLATE 16.

FIG. 6. Rabbit 30. Abdominal aorta. There is extensive calcification in the media, complete sloughing of the intima with ulcerations extending deeply into the calcified areas of the media, and a cellular thickening with round cell infiltration in the adventitia. Microphotograph. Hematoxylin and Van Gieson's stain. Bausch and Lomb obj. $\frac{2}{3}$, oc. 1.

FIG. 7. Rabbit 27. Abdominal aorta. The wall of the vessel is largely necrotic and extensively calcified, with the exception of a narrow zone next to the adventitia. In the center of the photograph the media is of about normal thickness, the intima has sloughed, and two zones of calcification are seen, one on the surface and one in the deep media, the intervening medial tissue being necrotic with little nuclear staining. At either side are deep ulcers extending almost through the media. There is hemorrhage in the adventitia. Microphotograph. Hematoxylin and Van Gieson's stain. Bausch and Lomb obj. $\frac{2}{3}$, oc. 1.

FIG. 8. Rabbit 27. Abdominal aorta. A shallow aneurysmal dilatation is present. Otherwise the condition is similar to that in Fig. 7. Microphotograph. Hematoxylin and Van Gieson's stain. Bausch and Lomb obj. $\frac{2}{3}$, oc. 1.

PLATE 17.

FIG. 9. Rabbit 30. Abdominal aorta. Extensive necrosis and calcification of the media with broad shallow ulcers. Note the marked round cell infiltration in the adventitia. Microphotograph. Hematoxylin and Van Gieson's stain. Bausch and Lomb obj. $\frac{2}{3}$, oc. 1.

FIG. 10. Rabbit 14. Cross-section of the carotid artery. Necrosis of the muscle is seen throughout a narrow zone of fairly uniform width in the upper media with crowding together of the elastic fibers into a compact layer. There is an irregular thinning of the wall with the formation of shallow pouchings.

There is no calcification. Microphotograph. Hematoxylin and Van Gieson's stain. Bausch and Lomb obj. $\frac{2}{3}$, oc. 1.

FIG. 11. Rabbit 17. Cross-section of the femoral artery. There is a zone of calcification in the upper media and at the right a second area of calcification and softening in the deeper media. Microphotograph. Hematoxylin and Van Gieson's stain. Bausch and Lomb obj. $\frac{2}{3}$, oc. 1.

PLATE 18.

FIG. 12. Rabbit 27. Cross-section of the carotid artery showing calcified plates in the upper media. Microphotograph. Hematoxylin and Van Gieson's stain. Bausch and Lomb obj. $\frac{2}{3}$, oc. 1.

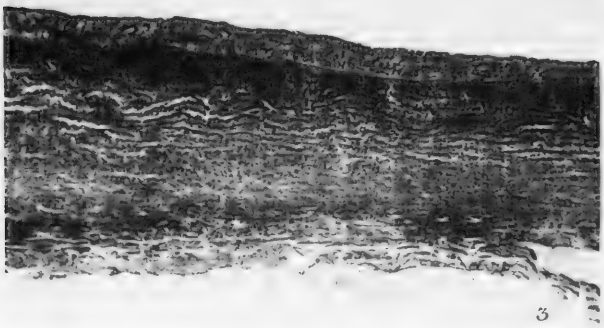
FIG. 13. Rabbit 17. Cross-section of a branch of the renal artery in the pelvis of the kidney, showing small calcified areas in the upper media. Microphotograph. Hematoxylin and Van Gieson's stain. Bausch and Lomb obj. $\frac{2}{3}$, oc. 1.

PLATE 19.

FIG. 14. Rabbit 10. Kidney. Lesions are present in all the glomeruli, consisting of partial necrosis of the tufts with the formation of fibrinous and hyaline masses. Many of the tubules contain casts. Microphotograph. Hematoxylin and Van Gieson's stain. Bausch and Lomb obj. $\frac{2}{3}$, oc. 1.

FIG. 15. Rabbit 27. High power of a typical kidney glomerulus, showing degeneration and necrosis of the tuft with hemorrhage and the formation of hyaline and fibrinous masses and a rich infiltration with polymorphonuclear leukocytes. Microphotograph. Hematoxylin and Van Gieson's stain. Bausch and Lomb obj. $\frac{1}{6}$, oc. 1.

(Bailey: Arteriosclerosis and Glomerulonephritis.)



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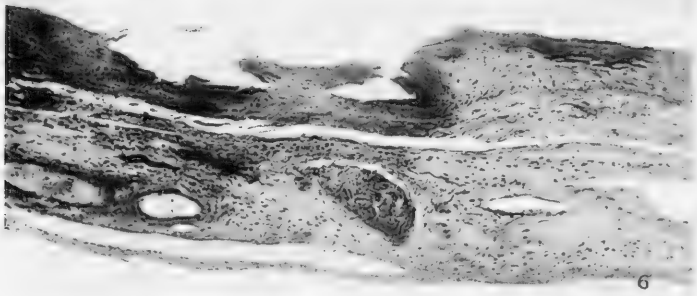
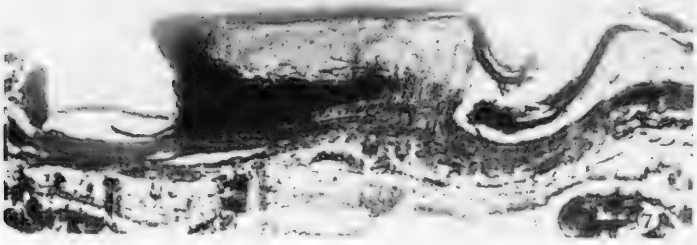
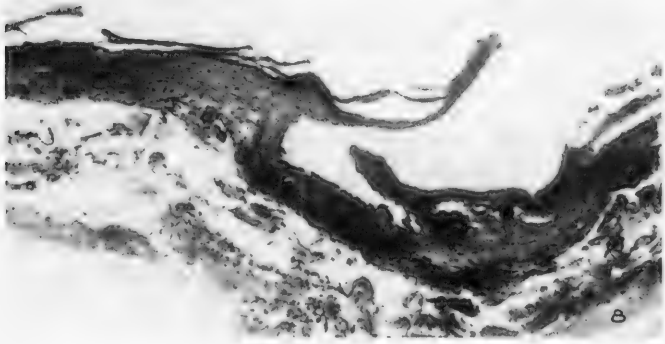
(Bailey: Arteriosclerosis and Glomerulonephritis.)







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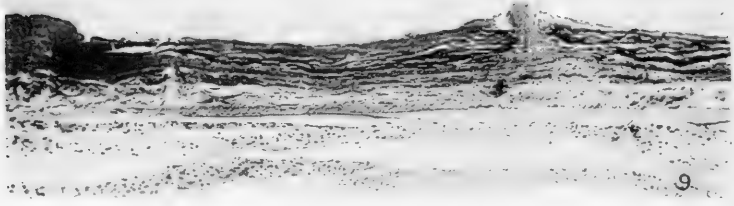

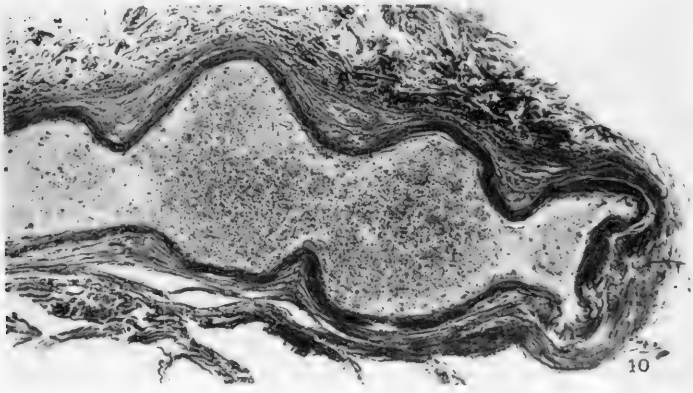

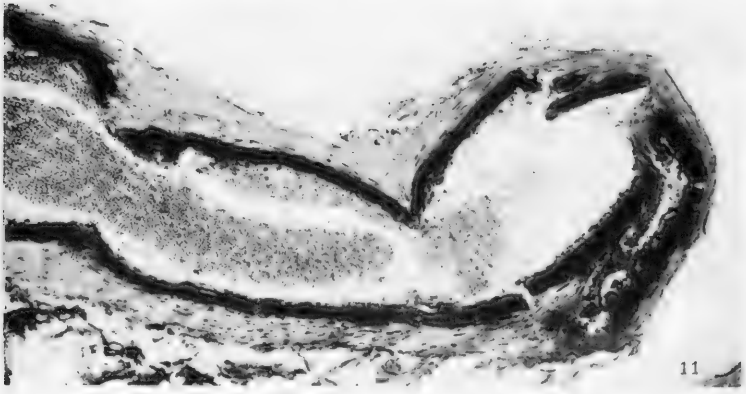

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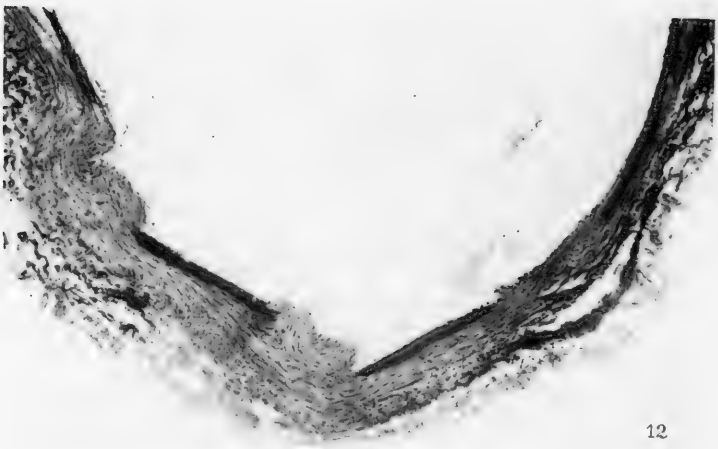

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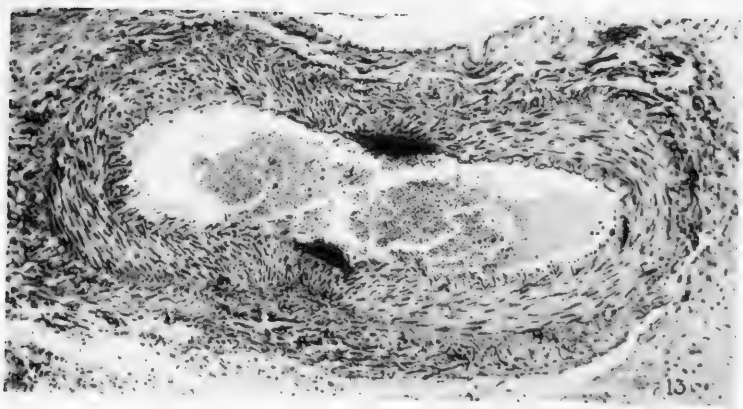

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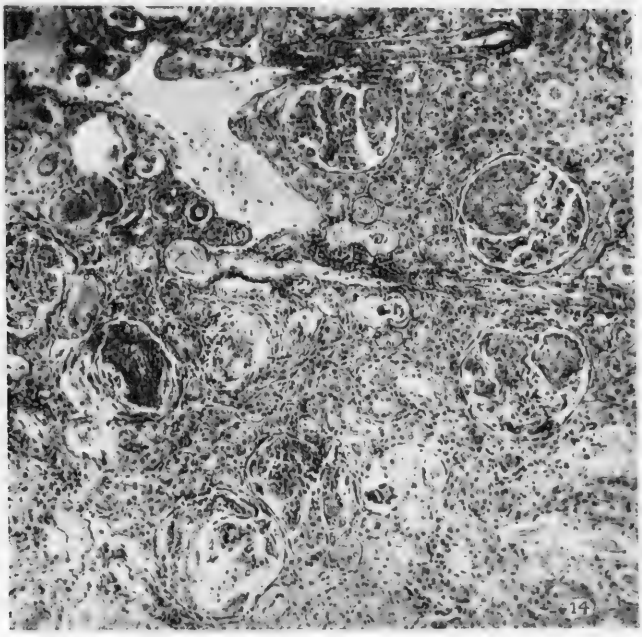
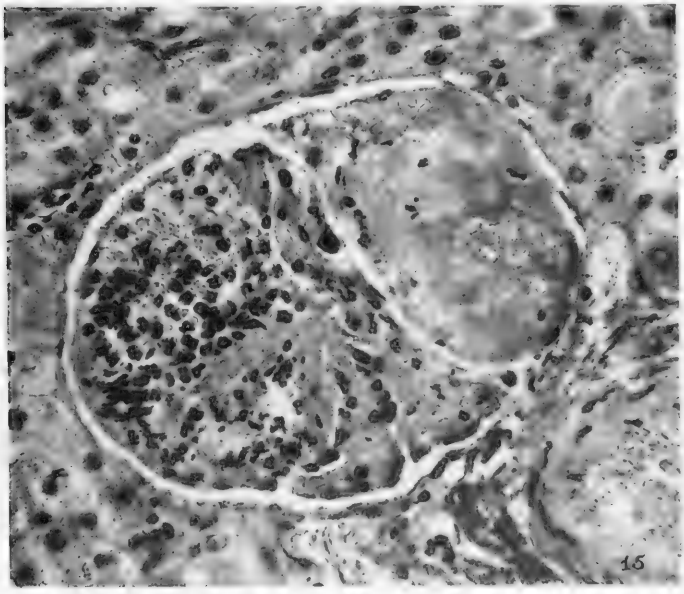
(Bailey: Arteriosclerosis and Glomerulonephritis.)





(Bailey: Arteriosclerosis and Glomerulonephritis.)

EXTIRPATION OF THE THYMUS IN THE GUINEA PIG.

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PLATES 20 AND 21.

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HISTORICAL.

In 1845 Restelli attempted to determine the function of the thymus by extirpating the gland from dogs, sheep, and calves. Since then more than twenty-five investigators have performed many series of extirpation experiments and extended their operations to more than fourteen species of animals, without obtaining results of sufficient agreement to bring the question of the effects produced by thymus extirpation to a final settlement. Some have found that removal of the thymus is without effect (Fischl, Pappenheimer, Nordmann, and others), and inferred that the thymus has either no function in postfetal life (Fischl) or none that can be demonstrated. Others have seen changes of a transitory character develop, and consequently have thought that the thymus exercises a transitory function corresponding to its own life history. Most prominent among these is Basch, whose experiments on dogs have been the models for subsequent investigators. Still others (Abelous and Billard, Matti, and Klose and Vogt) have determined that removal of the thymus is followed by symptoms and pathological changes of a profound character, culminating in death; and they have drawn the conclusion that the thymus is essential to life and assign to it an importance comparable to that of the adrenal, parathyroids, and pancreas.

The animals that have been used for extirpation experiments are the dog, rabbit, rat, guinea pig, frog (also tadpole), cat, fowl, pigeon, goat, sheep, calf, pig, and monkey; but some species are better adapted for thymus extirpation experiments than others. Basch is the authority for the frequently quoted statement that herbivorous animals are poor subjects, because very early in these animals bone has a high calcium content, which acts in a protective manner against the influences that result from the cessaton of thymus function. It is well known, however, that rickets occurs spontaneously in herbivorous animals (Jost and Koch), and numerous investigators have reported changes in the skeleton of the rabbit following thymectomy, and one (Klose) has obtained rachitic-like changes in goats. But no one has succeeded in producing alterations in the skeleton of the guinea pig by thymus extirpation—unless the slight retardation in growth, noted by Soli,

is to be regarded in that light. If Basch's observation is true, that deprivation of the thymus is less apt to be followed by bone changes in hervibora than in omnivora or carnivora, another explantion is that extirpation of the thymus in the former is almost invariably incomplete.

In order to make clear the anatomical relations of the thymus in the guinea pig which distinguish it from all other mammals and make complete removal impossible, it is first necessary briefly to trace the development of the thymus not only in the guinea pig but in other mammals.

Development of the Thymus in the Higher Mammals, with Especial Reference to Its Development in the Guinea Pig.

The higher mammals may be divided into three groups, according to the situation of the thymus (Hammar). In the first group the thymus is chiefly or entirely in the thorax; in the second, in both thorax and neck; and in the third, in the neck alone.

The thymus of most of the higher vertebrates is derived from the third pharyngeal pouch alone or from the third and fourth pouches, and is therefore purely entodermal in origin. The thymus of the swine (Zotterman, Badertscher) and of the mole (Schaffer and Rabl), on the other hand, takes origin not only from the third pharyngeal pouch but also from the ductus precervicalis medialis, a derivative of the ectoderm, and the thymus of the guinea pig also has a twofold origin from ectoderm and entoderm, as will be pointed out later.

The mammalian thymus appears very early as a cylindrical outgrowth in a ventral and mesial direction from the ventral diverticulum of the third pharyngeal pouch. Simultaneously with the appearance of this thymus anlage, called thymus III, there occurs a proliferation of the epithelium of the dorsal diverticulum and adjacent parts of the pouch which soon undergoes histological differentiation into the anlage of the larger of the two parathyroids, parathyroid III.

While thymus III and parathyroid III are forming from the ventral and dorsal diverticula of the pouch, the mesial portion, roughly that part of it between these structures and the pharynx, called the ductus pharyngobranchialis, narrows, atrophies, and disappears

altogether, so that thymus III and parathyroid III are freed from their connection with the pharynx, becoming independent structures. The thymus anlage elongates and is now termed the thymus cord.

In the species constituting the first group, of which man and the dog are representative, the caudal end of the thymus cord thickens and the cranial end becomes thinner. The entire cord then begins a migration in a caudal direction, its descent being associated with that of the great vessels and heart, and since the thick caudal portion moves at a more rapid rate than the thin cranial portion, the latter becomes drawn out into a thin strand. The thick caudal portion, continuing its migration, enters the thorax and, joining its fellow of the opposite side, forms the thoracic portion of the thymus. The thin strand representing the cranial portion of the cord atrophies and entirely disappears, except the lowermost part, which persists as a continuation of the thoracic portion into the base of the neck, forming the cervical process, or cornu of the completely developed gland. Parathyroid III, which remains connected with the upper end of the thymus cord, is carried caudally with the latter, but halts near the lower end of the thyroid anlage, assuming its permanent position near the lower pole of the thyroid.

In the second group of animals, which have both neck and breast thymus (the hoofed animals are examples), the caudal portion of the thymus cord enlarges and enters the thorax to form the thoracic thymus, exactly as has been described for the first group. But the remainder of the cord does not atrophy and disappear; indeed the cranial end of it hypertrophies, forming an enlargement, known as the head of the thymus, and in certain members of the group, at least (ox, horse, goat, and swine) moves in a cranial direction with the growth of the neck or, according to some embryologists, is caught in the loop of the hypoglossal nerve and drawn towards the cranium to a position opposite the bifurcation of the great vessels, well above its point of origin. Shortly before birth, or during the first year, the stem of the thymus, connecting cervical and thoracic portions, disappears, so that the two parts of the gland become separate.

In the guinea pig,[1] which forms the third group, the thymus anlage

[1] The development of the thymus in the guinea pig has been investigated by Maximow and Ruben. The account of its development is based on the work of Ruben.

develops from the whole of the third pharyngeal pouch with the exception of those parts of it which form parathyroid III and the neck. When set free together with parathyroid III from its connection with the pharynx through atrophy of the neck of the pouch, it does not elongate as in those animals destined to have a thoracic thymus, but assuming a rounded or oval form remains essentially in its original position. According to Ruben, the loop of the hypoglossal nerve cuts in between parathyroid III and the thymus mass, separating the former from the latter without carrying the latter upwards. A portion of the ectodermal precervical vesicle early becomes incorporated.

In certain species of animals a thymus anlage, first described by Groschuff, develops also at the fourth pharyngeal pouch together with the parathyroid of that pouch, and is known from its origin as thymus IV, or thymus metamere IV. When the neck of the fourth pharyngeal pouch disappears, thymus IV and parathyroid IV in their turn migrate, but in a mesial direction, to the lateral portion of the thyroid anlage, where they take their permanent position together. In several species parathyroid IV regularly becomes incorporated in the substance of the thyroid, and thymus IV also, if present. Thymus IV has been found in the cat, ox, sheep, horse, goat, rat, rabbit, bat, and more rarely in man and the dog (Groschuff, Kohn). In the guinea pig, however, it does not develop, and even parathyroid IV is frequently rudimentary or present only on one side, or is even absent altogether (Ruben).

Thus the thymus in the guinea pig, unlike the thymus in other mammals, remains a purely cervical organ[2] (Ruben, Maximow) and does not possess the accessory lobe derived from the fourth pharyngeal pouch so frequently seen in other species. It would seem, therefore, as if the guinea pig should be especially adapted for complete extirpation of the thymus. That this is not the case, however, will be shown later.

[2] Though the thymus of the mole has an apparent resemblance to that of the guinea pig in that it also is limited to the neck, the actual development in the two animals is different, because a thoracic thymus actually forms in the mole and subsequently atrophies, while in the guinea pig none ever forms.

Results of Previous Investigators Following Thymus Extirpation in the Guinea Pig.

At least four investigators have used the guinea pig for the study of the effects of thymectomy. Basch states that he has performed thymectomy experiments on guinea pigs, but he has left no record of them so far as can be found.

Vincent, in 1903, removed the thymus from a series of guinea pigs aged from 10 days to 1 month, but was unable to discover any effect on growth or any change in the blood.

Paton and Goodall, in the following year, performed extirpation experiments on the guinea pig. They found that deprivation of the thymus was followed by a decrease in the number of lymphocytes for about 2 months without coincident alteration in the other elements of the blood, but they observed no disturbance in growth and no variation of any kind from the normal.

In 1905, Paton published a new set of thymectomy experiments on guinea pigs, in which special study was made of the effects produced on the genital organs. His experimental material comprised twenty-four guinea pigs, 1 day to 1½ months old at the time thymectomy was performed, and killed at varying periods after operation. The control animals were selected from the same litters when possible, but when this was impossible (and he does not indicate how often this was) they were matched with the operative animals according to weight. Inasmuch as he had no record of the dates of birth of his animals he was compelled to estimate their ages from their weights. Since he found that sexual maturity in the guinea pig occurs at about the 3rd month, when the animal has reached a weight of approximately 300 gm., he assumed in his experiments that animals under 300 gm. were under 3 months of age and sexually immature, while animals over 300 gm. were over 3 months ot age and sexually mature. The first group of six guinea pigs (there were six controls) weighed between 75 and 145 gm. at the time of operation and were allowed to live from 2 to 6 weeks; they were killed at such times as to make the final weight of all members of the group lie between 100 and 200 gm. The average postmortem weight of the operative animals was 165 gm., of the controls 152 gm.; of the testes and epididymes of the operative animals, 0.23 gm.; of the testes and epididymes of the controls, 0.18 gm. The second group of sixteen animals (there were fourteen controls) weighed from 84 to 280 gm. when the thymus was removed, and were killed at the end of 4 weeks—in two instances 8 weeks—when the weights of all were between 200 and 300 gm. The average final weight of the operative animals of this group slightly exceeded that of the controls, but the average weight of the testes and epididymes of the thymectomized animals was 0.88 gm., while that of the controls was but 0.60 gm. The two groups just mentioned comprised the immature material of Paton's experiments. The third group, composed of but two operative animals (there were three controls), represented his entire mature material. One of the two, weighing 250 gm. at the operation, was killed at the end of 3 weeks,

weighing 310 gm. The other, weighing 280 gm. when operated upon, was allowed to live only 1 week. The testes, with epididymes attached, of these two animals weighed almost the same (1.24 gm.) as those of the three controls (1.33 gm.). Paton was unable to show that the loss of the thymus affected in any way the time of conception of female guinea pigs.

Though it seems questionable whether Paton could draw definite conclusions from his experiments, except perhaps such general ones as that removal of the thymus in the guinea pig is compatible with life, or that it does not produce any very gross changes, he drew the specific conclusion that the thymus inhibits the growth of the testes in immature but not in mature animals; and since Calzolari, Henderson, and others had already established the fact that the thymus continues to persist uninvoluted in animals castrated before maturity, he put forward the theory that prior to the period of sexual maturity thymus and sex glands act antagonistically, each exerting an inhibitory influence on the other.

In the same year (1905) and again in 1909 Soli performed thymectomy experiments on guinea pigs. The major part of his work, however, was done on fowls, in which he discovered that extirpation of the thymus resulted in well marked retardation in growth and development of the testes. He obtained similar results in the majority of fifteen guinea pigs, although they were much less marked than in the case of the fowls. Although Soli's results in guinea pigs appear to contradict the findings of Paton, they do so only to a limited extent, for all Soli's guinea pigs with the exception of two weighed more than 300 gm. when killed (Paton). (The maximum weight for immature animals in Paton's experiments was 300 gm.). The testes of Soli's two thymectomized guinea pigs, which weighed under 300 gm., were, however, slightly lighter than those of the controls.

In 1911 Paton returned to the problem of the relationship of thymic and testicular functions. Was it not possible that each organ exercised a primary, independent, stimulating influence on growth rather than an inhibitory influence on the other, as according to the theory he had previously advanced? To put this new hypothesis to the test, he first determined in a series of four guinea pigs and controls that castration alone before sexual maturity, *i.e.*, in animals under 300 gm., did not affect the weight. He next found by combined extirpation of both thymus and testes in a series of sixteen guinea pigs under 300 gm., that retardation in the growth of the animal measured in terms of weight did occur. Since his previous experimental work had shown that loss of the thymus alone had no influence on growth, he regarded the chain of experimental evidence necessary for the new hypothesis as complete. His final conception of the relationship of the two organs to each other was, therefore, that each stimulated growth in the immature animal independently of the other and consequently that the hypertrophy of the testes, when the thymus was removed, and the continued persistence of the thymus when the testes were removed, in sexually immature animals were compensatory in nature. On performing similar experiments with female guinea pigs he was unable to produce changes corresponding to those in the male.

It should be added that Paton used controls from "the same batches" of animals, and again that he had recourse to rough estimations of the ages from the weights.

Halnan and Marshall are the most recent investigators to attack the thymus problem in the guinea pig. They found that its removal in young guinea pigs, most of which weighed about 150 gm. did not affect growth, and that castration led to an arrested atrophy and subsequent hypertrophy of the thymus, and, more important, as bearing on Paton's work, that simultaneous removal of the testes and thymus did not affect the growth of immature animals, and thymectomy was not followed by hypertrophy of the testes. Thus they entirely failed to confirm the results of Paton. Their paper concludes with a note from Yule showing by methods of statistical analysis that the differences which Paton found in the testes of thymectomized and control guinea pigs were not greater than could be explained on the basis of chance variation.

Paton and Goodall remark: "The chief obstacle to the investigation of the functions of the thymus is the difficulty of its removal on account of the situation of part of it, in most animals, in the thorax. We were fortunate enough to begin our investigations upon guinea-pigs and we found that in these animals the structure is entirely cervical." Halnan and Marshall speak of thymectomy in the guinea pig as a simple operation. Klose and Vogt, on the other hand, describe thymectomy in the guinea pig as a dangerous operation which must be done in two stages and frequently causes death from hemorrhage. The writer selected the guinea pig for his experiments.

EXPERIMENTAL.

The operation for the removal of the two main lobes of the thymus in the guinea pig is simple, owing to their superficial position, for they lie one on either side of the median line of the neck, just beneath the platysma and deep fascia about midway between the tip of the lower jaw and the upper end of the thorax, a little in front of, but almost opposite the angle of the jaw. A median incision about 0.5 cm. long, with its middle point a little anterior to the angle of the jaw, is carried to the deep fascia, and when the edges of the wound are pulled apart the two flat, oval-shaped, granular-looking lobes of the thymus can be plainly seen shining through, having a paler color than the pink salivary glands which lie near them. Each lobe in the well nourished new-born guinea pig weighing 100 gm. measures from 10 to 15 by 7 to 10 mm. The deep fascia is then incised and each lobe in turn lifted up and freed from the salivary glands by blunt dissection. As these latter are pushed away a sheet of transparent fascia

is exposed, connecting the thymus with the neck, and in this sheet of fascia two blood vessels, larger than the rest, can be seen, one entering the lobe a little behind the anterior pole, the other a short distance in front of the posterior pole. In the earlier experiments these vessels were carefully tied off, but in the later experiments they were burned through, together with the fascial sheet, by means of a hot platinum wire. At this point in the operation great care is necessary not to leave accessory lobes of thymus, which become visible only as the main lobes are lifted up, lying near but unattached to the main lobe or close to the salivary gland, in the fascial sheet mentioned. If the main lobe of the thymus is removed first with the expectation of excising the accessory lobes later, the latter are found to fall back into the cavity of the wound the moment tension is released, and are lost. It is necessary therefore to cut or burn through the fascial sheet as far away from the main lobe as possible in order to secure the accessory lobes with the main lobes, and often to remove also fairly large parts of the salivary glands, if the latter cannot be easily separated. Frequently as many as two accessory lobes are present on each side, not larger than pin heads.

The Discovery, by Serial Section, of Accessory Lobes of the Thymus in Close Relation to the Parathyroids.

The operations were conducted under ether anesthesia. Little preparation aside from shaving the operative field and cleansing with alcohol was necessary, for the animals never became infected. As soon as they had recovered from the anesthesia they appeared to be well. A number of the guinea pigs died a short time after the operation but not as the result of it, for the mortality was equally high among the controls.

The animals were kept under observation for variable lengths of time, and were then killed. At autopsy the tissues of the neck of all the thymectomized animals were taken out *en masse* to be studied in serial section for thymus rests. As the block of tissue was removed from each animal it was turned over on its under surface, and first the thyroid, then the larynx and trachea, were dissected away, the former for finer histological study than would otherwise have been possible, the latter to facilitate serial section cutting. Great care

was taken to carry away with the trachea and thyroid as little other tissue as possible.

When the serial sections from the cervical tissues of nine of the thymectomized guinea pigs were studied, it was found that thymus rests near the parathyroid were present in six of them; in the remaining three no thymus tissue could be discovered. In these three thymus-free animals, however, no parathyroid tissue could be found, and inasmuch as the only conceivable explanation for the absence of the parathyroids was that they had been lost when the thyroids and trachea were removed, the possibility became apparent that accessory lobes of thymus lying near the parathyroid might also have been there and been lost with the latter. In the cervical tissues of two of the animals which had thymus rests near the parathyroid, accessory thymus tissue at some distance from the parathyroids was also found.

Since it seemed scarcely worth while to investigate the mutilated cervical tissues of the remaining thymectomized guinea pigs in order to throw further light on the occurrence of accessory lobes of thymus tissue, the intact cervical tissues, including trachea and thyroid, of five additional guinea pigs were examined in serial section. Of these five guinea pigs, four came from the same litter, and of the four, two had been freshly thymectomized. In all five animals accessory lobes were found in proximity to the parathyroids, and in three of them additional accessory lobes not connected with the parathyroids and too far removed from the main body of the thymus to have been reached at operation.

Although the number of guinea pigs examined is too small to permit any general conclusions, it seems probable that accessory lobes of thymus occurring in relation to the parathyroids are usual in the guinea pig and that other accessory lobes too remote from the main lobes of the thymus for removal with the latter are not uncommon.

The Relations and Characters of the Accessory Lobes of the Thymus Found in the Guinea Pig after Supposed Complete Thymectomy.

The accessory thymus tissue which was found in relation to the parathyroids was in the form of lobes having well defined cortex and medulla, and in short a histological appearance identical with that of the main lobes. In some instances only a single lobe could be found

in relation to a parathyroid, but in other instances there appeared to be several separate lobes clustered around it; though when certain sections seemed to indicate the presence of several separate masses of thymus, the study of the other sections sometimes showed that in reality a single lobulated mass of thymus tissue existed. In some of the animals the accessory lobe of the thymus, or the largest of them if several existed, was two to three times the size of the parathyroid, but in a number of the animals the accessory thymus lobe was about the same size as the parathyroid or even smaller. In at least half the animals thymus tissue and parathyroid were fused together, or at least one passed directly into the other. In one animal the parathyroid on the right side was drawn out into a thin strand which was much longer than the thyroid and extended considerably in front of it; it terminated in a small thymus lobe, so shaped that it would have been regarded as the continuation of the parathyroid but for its different structure (Fig. 2). In sections from another animal, thymus and thyroid tissue were so intermingled at one point that there was great difficulty in distinguishing them, while at another point in the sections an island of thymus surrounded by parathyroid tissue could be seen (Figs. 3 and 4). In the animals in which thymus tissue did not actually join parathyroid, it was situated close to it, never much farther away than the thickness of the parathyroid.

Of course in the mutilated cervical tissues of the six thymectomized animals first studied, it was not possible to determine more than the size and relations of the accessory lobes with reference to the accompanying parathyroid, but it was possible to obtain additional and more precise information from the study of the intact cervical tissues of the five guinea pigs killed for that purpose. In them it was found that the accessory lobes of thymus occurred on both sides in only three animals, though they occurred on one side in all five, as already stated. It was also clear that the parathyroid which the accessory lobes accompanied was the one from the third pouch. The identity of this parathyroid could be determined (1) by its larger size, and (2) by its position with reference to the thyroid, for in all five animals it lay on the outer side of the thyroid, in three instances separated some distance from the latter. In most of the animals it was in proximity to a comparatively large blood vessel, supposed to be a thy-

roid artery. In one animal the accessory lobe lay on one side of this vessel, the parathyroid on the other. On the other hand, parathyroid IV, which was much smaller than parathyroid III, lay close to the thyroid on its inner surface in all but one instance, and in three animals was partially embedded in its substance. It was absent on one side in three animals, and so small as to be barely visible in one. Accessory lobes of thymus were not found in association with this parathyroid (Fig. 1).

In this connection it is worth noting that no constant relationship in the positions of the two parathyroids in the cranial caudal direction existed. In some of the guinea pigs parathyroid III was anterior, in others posterior to parathyroid IV. In one animal parathyroid IV was in front of parathyroid III on one side and behind it on the other. The commonest arrangement seemed to be for parathyroid III to lie near the anterior end of the thyroid and parathyroid IV near the middle or posterior part. In the mammals in which the thymus migrates caudally, it will be remembered that parathyroid III is carried caudally with the thymus so that it comes to be the more caudally situated of the two parathyroids. The absence of this relationship in the guinea pig is easily explained by the failure of the thymus to migrate in that animal.

In addition to the accessory lobes already described as occurring in intimate relationship with the parathyroids, accessory lobes were found in two of the animals at some distance from the parathyroid in the neighborhood of the main thymus lobes, but they were probably too far separated from them to have been removed at operation.

The existence of accessory lobes of thymus derived from the third pharyngeal pouch lying in close relation to the parathyroids, has never before been described in the guinea pig, as far as the writer is aware, but their occurrence in other species has been known for some time. They are usually present in the rabbit, cat, and rat (Kohn), and have been found in the dog and in man (Ammann, Kürsteiner). The study of the development of the thymus makes their presence and also their close connection with parathyroid III easy to understand, for, as already pointed out, thymus and parathyroid take origin coincidently in the closest proximity from the third pharyngeal pouch, are freed at the same moment by the atrophy of the neck of the pouch, and, if

migration occurs, migrate with each other. To explain the presence, therefore, of an accessory lobe of thymus in close connection with the parathyroid it is necessary merely to suppose that a part of the original thymus anlage adjacent to parathyroid III adhered to the latter at the time of their separation. If the theory of Ruben is correct that in the guinea pig parathyroid III is separated from the thymus mass through the agency of the loop of the hypoglossal nerve in its passage upward, it would be necessary simply to assume that the nerve had cut off a small portion of the main mass of the thymus with the parathyroid instead of accurately dividing the one from the other.

The practical significance of the discovery of these minute accessory lobes of thymus in such intimate relation to the parathyroids is that the thymus can only rarely be completely extirpated in the guinea pig. Almost all, if not all, thymectomies in this animal have been, therefore, partial thymectomies, and the positive experimental findings of Soli and Paton must be interpreted in that light.

The Value of Incomplete Extirpation of the Thymus as a Means of Determining Thymus Function.

The question naturally suggests itself whether incomplete extirpations of the thymus have any value as a means for determining thymus function. Since it is well established that reduction of certain organs of internal secretion, for instance the adrenal, thyroid, and parathyroid, to minute rests causes well marked signs of insufficiency, it might reasonably be supposed that sudden reduction of the thymus to a small rest in a newly born animal would make itself known, if it plays the important part in metabolism attributed to it by Matti and Klose. And the literature contains considerable evidence that this is the case. Some of Basch's[3] extirpations in dogs seem to have been incomplete. Fulci obtained marked rachitic changes in a rabbit by means of thymectomy, although a thymus rest almost as large as the normal gland was found at autopsy. Flesch discovered that incompletely thymectomized rats died in a manner indistinguishable from the completely thymectomized animals, with but a single excep-

[3] See Basch, K., *Wien. klin. Woch.*, 1903, xvi, 893.

tion. Though Klose and Vogt criticize the validity of a large part of the negative experiments of others by assuming that the thymectomies were incomplete, they accept the experimental work of those who have obtained results similar to their own, passing over the fact that the larger part of these thymectomies would be shown to be incomplete if subjected to the analysis which they applied to the other experiments. But recently Klose has reported rachitic changes in swine and goats induced by thymectomy, although complete thymectomy is impossible in both species, as he admits. To the group of investigators who have obtained positive results by means of partial thymectomy may now be added the names of Paton and Soli.

Doubtless many of the extirpations of the thymus which have been regarded as complete and reported as such in the literature in reality were partial extirpations, for it is impossible to be certain at the time of operation that a branching, friable organ like the thymus of the larger animals that have been used for extirpation experiments has been completely dissected out of the thorax and neck, especially considering the possibility of the accessory lobes. Likewise the failure to discover thymus rests at autopsy by inspection of the neck and thorax or microscopical section of bits of thymus tissue falls far short of proof that no thymus rests were actually there. The painstaking researches of Grosser and Betke have shown that in children accessory thymus lobes occur in the neck with a frequency never before suspected. If the thoracic and cervical tissues of larger animals that have been thymectomized, such as the dog and rabbit, could be studied in serial section, as was done by Pappenheimer in the rat, and by the writer in the guinea pig, it might be shown that complete thymectomy was rarely accomplished. Klose's statement that the most minute thymus rest left at operation regenerates until it reaches a size approximating that of the intact gland is not true. The literature seems to furnish, therefore, numerous instances of the effectiveness of partial thymectomy, and particularly, when the positive results of the partial thymectomies in guinea pigs of Paton and Soli are considered, justifies the publication of the following partial thymectomy experiments. But the actual problem is not whether partial thymectomy can produce results identical with complete thymectomy, but whether thymectomy, partial or complete, actually causes the changes which have been ascribed to it.

Results of Thymus Extirpation.

Fifty-five guinea pigs were thymectomized in the manner described, on the 1st, 2nd, 3rd, or 4th days of life, and in all but two instances were controlled from the same litter and sex. But since the mortality among such young animals was high, from trampling, inanition, and other causes, a large number of the experiments were interrupted at the outset. If the control died at the beginning of an experiment a guinea pig differing only a day or two in age was supplied in its place from another litter and the experiment continued. Later two epidemics of dysentery were encountered, which caused a number of fatalities and still further reduced the material, leaving eighteen thymectomized and sixteen control guinea pigs, which form the basis of this report. Twelve of the eighteen thymectomized animals had their original controls, and form, therefore, a complete series for the study of the effects of thymectomy from all standpoints. The remaining six thymectomized and four control animals lost their mates too late in the course of the experiments for anything to be gained by pairing them with each other or with fresh animals from other litters. Accordingly the six operative animals, with the four normal animals, are grouped separately as imperfect experiments which have value for the histological study of the organs but not for the study of the effects of thymectomy on growth or development.

All the animals were confined, in pairs, in wire cages about $1\frac{1}{2}$ feet long by 1 foot broad in a well lighted room, and were fed a diet of greens and oats each once daily. They were inspected frequently and systematically examined at the weighing times, which appear on the weight charts, for the various changes which have been reported in animals deprived of the thymus. One operative animal and two controls died spontaneously at the conclusion of the experiment, but the others were killed with ether. The bodies of all were autopsied at once, the stomach and intestines with their contents removed and weighed separately in order to determine the actual body weights of the animals (minus the weight of the food), and the weights of the different organs were taken. The tissues were preserved in formalin and the sections stained with hematoxylin and eosin. The growth of the thymectomized animals was normal.

The weight curves of the twelve operative animals and their controls were compared in pairs, but without demonstrating any preponderant variation at any stage of development, although there was considerable variation in the weight of the pairs of animals considered separately. In some unpublished experiments by Auchincloss and the writer guinea pigs were obtained prematurely by Cæsarean sections and thymectomized at once without causing any alteration in growth or development. The series of weights in two of these experiments are appended to the table of weights of the animals of the writer's own experiments (Table I).

The strength and activity of the operative animals also appeared to be normal. As the two guinea pigs constituting each experiment were caged together, it was possible to form some idea of their relative strength by observing which drove about the other. In some instances it was the control, in others the operative animal that had the mastery.

The evidence of sexual maturity appeared to be unaffected by thymectomy. This observation was made in the experiments of Auchincloss and the writer already referred to, in which it was found that the testes of thymectomized and control males descended at about the same time and that several pairs of males and females thymectomized soon after birth, caged separately, and allowed to breed in competition with control pairs of intact males and females of the same age, produced young at about the same age.

No trophic disturbances of the hair or abnormality in the growth of the teeth were noted in the operative animals.

At autopsy the fat appeared to be equally distributed in both sets of animals, and the viscera of the thymectomized animals did not differ in their gross appearance from the viscera of the controls. The weights of the animals and of their organs are given in Table II. Although there is exhibited the greatest individual variation in the weights of the organs, there seem to be no constant differences in the two groups. The most interesting feature brought out in the table is the relatively large weight of the gastric and intestinal contents in the guinea pig. It will be noted that the excised stomach and intestines and contents of the guinea pigs—they were killed at various times of the day—weighed between 38 and 160 gm., with an average

TABLE I.

Weight of Thymectomized and Control Guinea Pigs.

No. and sex.	Age at operation.	Age at death.	Killed or died.	Record of weights in gm.								
	days	*mos. days*										
				1915 Mar. 2	*1915* Mar. 9	*1915* Mar. 23	*1915* Apr. 6	*1915* Apr. 27	*1915* May 11	*1915* May 17		
1* ♀	1	2 15	Died.	50	90	140	190	220	210	190		
1C ♀	1	2 15	"	40	80	150	250	185	190	140		
				1915 Mar. 2	*1915* Mar. 9	*1915* Mar. 23	*1915* Apr. 6	*1915* Apr. 27	*1915* May 11	*1915* May 17		
2* ♀	1	2 15	Killed.	60	120	165	150	200	290	266		
2C ♀	1	2 15	Died.	40	80	150	250	185	190	140		
				1914 May 14	*1914* July 8	*1914* Aug. 11	*1914* Sept. 4	*1914* Oct. 20	*1914* Dec. 4			
3* ♀	4	6 20	Killed.	158	270	540	480	560	560			
3C ♀	4	6 20	"	122	200	425	300	420	500			
				1914 Mar. 28	*1914* Apr. 5	*1914* June 6	*1914* July 8	*1914* Aug. 11	*1914* Sept. 4	*1914* Oct. 20	*1914* Nov. 20	
4* ♀	3	7 25	"	121	132	320	490	540	525	580	520	
4C ♀	3	7 25	"	115	129	370	460	550	505	695	530	
				1914 May 14	*1914* June 6	*1914* July 8	*1914* Aug. 11	*1914* Sept. 4	*1914* Oct. 20	*1914* Nov. 20		
5* ♀	3	6 7	"	120	190	265	380	290	375	385		
5C ♀	3	6 7	"	122	195	285	400	340	445	450		
				1914 May 14	*1914* June 6	*1914* July 8	*1914* Aug. 11	*1914* Sept. 4	*1914* Oct. 20	*1914* Dec. 4		
6 ♀	3	6 19	"	132	215	360	435	345	485	552		
6C ♂	3	6 19	"	152	235	370	510	455	585	607		

				1914	*1914*	*1914*	*1914*	*1914*	*1914*	*1914*		
				May 20	June 6	July 8	Aug. 11	Sept. 4	Oct. 20	Dec. 15		
7* ♀	4	6	25	Killed.	175	180	252	365	302	430	440	
7C ♀	4	6	25	"	180	200	220	305	280	380	380	
					1914	*1914*	*1914*	*1914*	*1914*	*1914*		
					June 4	July 8	Aug. 11	Sept. 4	Oct. 14	Dec. 14		
8 ♀	1	6	8	"	85	240	335	290	475	430		
8C ♀	1	6	8	"	72	220	310	200	300	375		
					1914	*1914*	*1914*	*1914*	*1914*	*1914*		
					June 18	July 8	Aug. 11	Sept. 4	Oct. 14	Dec. 21		
9* ♀	1	6	4	"	110	160	235	280	405	410		
9C ♀	1	6	4	"	115	150	225	190	315	310		
					1915	*1915*	*1915*	*1915*	*1915*	*1915*	*1915*	*1915*	*1915*
					Jan. 28	Feb. 9	Feb. 22	Mar. 9	Mar. 23	Apr. 6	Apr. 27	May 11	May 18
10* ♂	1	3	20	"	75	130	230	220	290	315	355	370	330
10C ♂	1	3	20	"	70	120	220	220	310	325	390	390	355
					1915	*1915*	*1915*	*1915*	*1915*	*1915*	*1915*	*1915*	*1915*
					Feb. 3	Feb. 9	Feb. 22	Mar. 9	Mar. 23	Apr. 6	Apr. 27	May 11	May 19
11* ♀	1	3	17	"	50	100	190	230	270	325	350	440	425
11C ♀	1	3	17	"	70	135	230	280	325	290	310	480	460
					1915	*1915*	*1915*	*1915*	*1915*	*1915*	*1915*	*1915*	*1915*
					Feb. 6	Feb. 9	Feb. 22	Mar. 9	Mar. 23	Apr. 6	Apr. 27	May 11	May 19
12* ♀	1	3	14	"	70	75	190	220	240	290	300	350	310
12C ♀	1	3	14	"	60	75	155	210	270	315	325	370	335
A† ♂		4‡		Killed.	103	103	250	300	345	420	575	600	
AC ♀				"	120	180	260	370	410	440	485	500	
B* ♂		5½		"	68	100	177	225	300	375	385	435	475
BC ♂				"	58	97	172	225	280	356	390	455	480

* The operative and control animal were from the same litter.

† From unpublished experiments of Auchincloss and Park.

‡ Born by Cæsarean section.

TABLE II.

Body Weight and Weight of Organs in Thymectomized and Control Guinea Pigs.

No. and sex.	Age.		Killed or died.	Body weight.	Weight of gastro-intestinal tract with contents.	Body weight without gastro-intestinal tract and contents.	Weight of organs.								
							Liver.	Kidneys.	Heart.	Pancreas.	Testes.	Spleen.	Adrenals.	Thyroids.	Thymus.
	mos.	*days*		*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1* ♀	2	15	Died.	190	46	144	7.5	2.1	0.95	0.65		0.34	0.25	0.01	
1 C ♀	2	15	"	140	38	102	5.8	1.7	0.5	0.57		0.6	0.2	0.05	0.16
2* ♀	2	15	Killed.	265	85	180	13.5	1.4	0.8	1.05		0.32	0.25	0.07	
2 C ♀	2	15	Died.	140	38	102	5.8	1.7	0.5	0.57		0.6	0.25	0.05	0.16
3* ♀	6	20	Killed.	560	141	419	21.5	3.4	1.8	1.6		0.67	0.32	0.1	
3 C ♀	6	20	"	500	113	387	25.3	4.2	2.23	1.7		0.79	0.36	0.05	0.3
4* ♀	7	25	"	520	136	384	38.0	5.52	2.32	1.67		0.92	0.33	0.065	
4 C ♀	7	25	"	530	160	370	48.1	5.0	1.8	1.2		0.92	0.41	0.038	0.2
5* ♀	6	7	"	385	84	301	26.0	3.67	1.58	1.2		0.42	0.33	0.03	
5 C ♀	6	7	"	450	115	335	21.0	3.7	1.9	1.5		0.46	0.21	0.025	
6 ♀	6	19	"	552	126	426	37.5	6.8	1.9	1.77		0.82	0.4	0.1	
6 C ♂	6	19	"	607	127	480	34.6	4.9	2.3	1.89	1.66	0.62	0.28	0.11	0.21
7* ♀	6	25	"	440	134	306	19.5	3.15	1.1	1.0		0.55	0.26	0.01	
7 C ♀	6	25	"	380	119	261	17.0	3.0	1.05	1.15		0.43	0.3	0.03	0.13
8 ♀	6	8	"	430	121	309	26.2	3.7	2.0	1.7		0.7	0.45	0.05	
8 C ♀	6	8	"	375	85	290	24.0	4.0	1.7	1.5		0.6	0.37	0.05	0.35
9* ♀	6	4	"	410	104	306	31.5	4.1	1.48	1.38		0.45	0.35	0.1	
9 C ♀	6	4	"	310	83	227	28.0	3.2	1.45	0.8		0.4	0.35	0.1	0.2
10* ♂	3	20	"	335	95	240	13.7	2.7	1.0	1.05	2.6	0.45	0.3	0.01	
10 C ♂	3	20	"	355	97	258	15.5	3.3	0.8	1.2	2.31	0.3	0.3	0.05	0.2
11* ♀	3	17	"	425	87	338	12.7	3.1	1.21	1.21		0.31	0.21	0.08	

11	C	♀	3	17	Killed.	460	101	359	13.4	3.61	1.36	1.05		0.56	0.25	0.06	0.55
12*		♀	3	14	"	310	67	243	12.61	2.81	0.85	1.11		0.35	0.2	0.07	
12	C	♀	3	14	"	335	95	240	13.4	3.11	1.1	1.1		0.36	0.2	0.05	0.4
13†	N	♀	3	8	Killed.	410	90	320	10.4	2.55	1.15	1.2		0.5	0.3	0.05	0.2
14		♂	3	13	"	230	58	172	9.0	2.01	1.0	0.81	1.21	0.15	0.21	0.01	
15	N	♂	3	19	"	370	132	238	14.0	3.0	1.05	1.15	2.8	0.31	0.21	0.05	0.16
16		♀	5	10	"	560	105	455	19.8	4.3	1.4	1.3		0.85	0.4	0.1	
17	N	♀	6	14	"	455	94	361	36.5	4.1	1.5	1.6		0.62	0.32	0.12	0.2
18		♂	7	4	"	475	97	378	13.5	4.01	1.15	1.21	3.8	0.45	0.31	0.1	
19		♀	8	13	"	512	127	385	33.7	4.2	2.13	1.55		0.83	0.4	0.12	
20		♂	11		"	525	152	373	26.46	4.82	2.17	1.52	2.62	0.62	0.35	0.06	
21		♂	11	9	"	605	126	479	39.4	5.16	2.5	1.25	3.5	0.66	0.55	0.15	
22	N	♂	11	22	"	595	131	464	28.5	4.4	2.4	1.8		0.5	0.27	0.04	0.6

* Both animals were from the same litter.

† This group of six thymectomized and four normal animals contains single survivors of experiments. The thymectomized members of the group have been used for the study of the effects of the removal of the thymus on the organs, and, for this purpose, the normal animals may be regarded as controls.

of 103 gm., amounting in most cases to about one-fourth of the total weight of the animal. If the weights of the gastro-intestinal tracts and actual body weights (body weight minus weight of gastro-intestinal tract) are compared, it will be seen that the living healthy guinea pig, fed in the usual way, is composed of 2 to 5 parts of body and 1 part of food.[4]

No histological changes, which were constant, could be found in any of the organs of internal secretion or in the bones. There were no signs of rickets. The femora and ribs cut before decalcification with the same stony resistance as the bones of the controls, and on microscopical examination showed no osteoid zone bordering the trabeculæ or irregularity in the growth of the cartilage. The thyroids displayed marked individual differences, as has been noted in other animals by different observers, but no variation common to one set of guinea pigs. The adrenals presented no constant differences such as have been described by Matti in the dog and also by Klose. Measurements of the cortex and medulla of the adrenals of the pairs of animals showed how variable the thickness of the medulla is in the guinea pig. Where the adrenal is flat and broad, the medulla is scarcely more than a line in thickness; where the adrenal has a more rounded shape, the medulla may have a diameter of 3 mm. The cortex measured uniformly about 1 mm. in thickness. Histologically the adrenals, hypophyses, and ovaries, and the testes of the few males in the experiments, were normal.

Although the negative experiments just described furnish no evidence for or against Paton's conclusion that extirpation of the thymus in immature guinea pigs causes the testes to hypertrophy, yet there are reasons why Paton's work should not be accepted on its face value. His problem concerned growth, and in the investigation of a problem of that nature the cardinal principle is to plan the experiments so as to insure absolute parallelism between each pair of experimental and control animals, and the value of the results obtained depends largely on the strictness with which this principle has been carried out. But Paton chose his controls largely from the same batches of animals and guessed at the ages from the weights, though exact correspondence

[4] The error of estimating the age of the guinea pig from the weight should be considered.

in age between each pair of animals was of vital importance to the success of his experiments. And, finally, he made no allowance for the weight of the gastric and intestinal contents in his determinations of the final weights of his animals. These considerations do not prove Paton's results wrong, but they show that his methods were inaccurate and make corroboration of his work necessary. Halnan and Marshall have repeated Paton's work and failed to corroborate it. The changes which Soli found in the testes of guinea pigs after thymectomy were so slight that his experiments in that animal may be considered negative. In the light of these facts it seems proper to regard the thymectomy experiments on guinea pigs of all investigators up to the present time negative.

Whether the failure to obtain changes in the guinea pig by means of thymectomy indicates that the thymus has no function in that animal gross enough to be brought out in that way, or whether it signifies that the reduction of the thymus to the accessory lobes is insufficient to excite signs of thymus insufficiency in the guinea pig is a question which cannot be answered in the present state of our knowledge; and to speculate in regard to it is idle, until the principle that thymus function can be demonstrated by thymus extirpation has been firmly established in other animals in which complete or more nearly complete removal of the thymus is possible.

CONCLUSIONS.

1. Accessory lobes of thymus, derived from the third pharyngeal pouch, occurring in close association with the parathyroids from the third pouch, were found in serial section of the cervical tissues of eleven out of fourteen guinea pigs, and probably would have been found in all fourteen but for a technical error.

2. It is probable, therefore, that accessory lobes of thymus having this situation and origin are usually, if not always, present in the guinea pig.

3. Additional accessory lobes of thymus belonging to, but at some distance from the main lobe were also present in several of the animals.

4. The discovery of these accessory lobes makes it certain that the guinea pig is unsuitable material for complete thymectomy, and prob-

ably complete extirpation of the thymus in this animal is rarely, if ever accomplished.

5. The extirpation experiments of previous investigators in the guinea pig must now be regarded as partial extirpations, and their results interpreted in that light.

6. Extirpation of the thymus in the guinea pig produced no changes in the writer's experiments.

7. The study of the serial sections of the cervical tissues of the guinea pig indicates that Ruben's statements regarding the parathyroid derived from the fourth pharyngeal pouch in the guinea pig are correct,—that it is much smaller than parathyroid III, may be rudimentary, and is sometimes absent at least on one side.

8. No accessory lobe of thymus was found accompanying the parathyroid from the fourth pouch, a finding also bearing out Ruben's statement that no thymus anlage springs from the fourth pouch in the guinea pig.

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EXPLANATION OF PLATES.

Plate 20.

Fig. 1. The right thyroid is shown with the parathyroid derived from the fourth pharyngeal pouch imbedded in its substance, and the parathyroid derived from the third pouch accompanied by an accessory lobe of thymus, lying on its outer border. *a*, accessory lobe of thymus; *b*, parathyroid from the third pouch; *c*, right thyroid; *d*, parathyroid derived from the fourth pharyngeal pouch.

Fig. 2. The parathyroid derived from the third pharyngeal pouch appears as a thin strand, which terminates anteriorly in an accessory lobe of the thymus also derived from the third pouch. The strand of parathyroid tissue measures two and one-half times the length of the accessory thymus lobe; less than half of it is included in the photograph. The thyroid does not appear as it lies too far posteriorly. *a*, small accessory lobe of thymus; *b*, larger accessory lobe; *c*, parathyroid continuous with thymus lobe; *d*, salivary glands; *e*, cartilage of trachea.

Plate 21.

Fig. 3. Accessory lobes of thymus are shown clustering around the parathyroid from the third pouch. Two separate masses of parathyroid are shown which, in serial section, could be seen to be continuous. In the larger of the two masses a small island of thymus can be seen. A study of the other sections shows it

not to be an island, but an arm of thymus tissue thrust into the parathyroid. In the smaller of the two masses of the parathyroid there is thymus tissue and also a duct-like space, lined with cylindrical epithelium. *a*, accessory lobes of thymus derived from the third pouch; *b*, parathyroid from the third pouch; *c*, duct-like space lined with cylindrical epithelium.

FIG. 4. The same parathyroid and group of accessory thymus lobes as shown in Fig. 3, but at a different level. *a*, thymus from the third pouch; *b*, parathyroid from the third pouch; *c*, duct-like space lined with cylindrical epithelium.

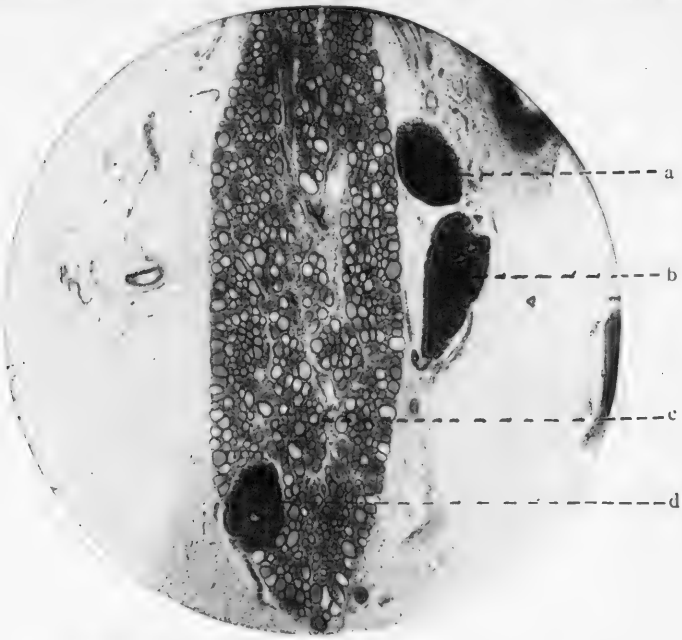

FIG. 1.

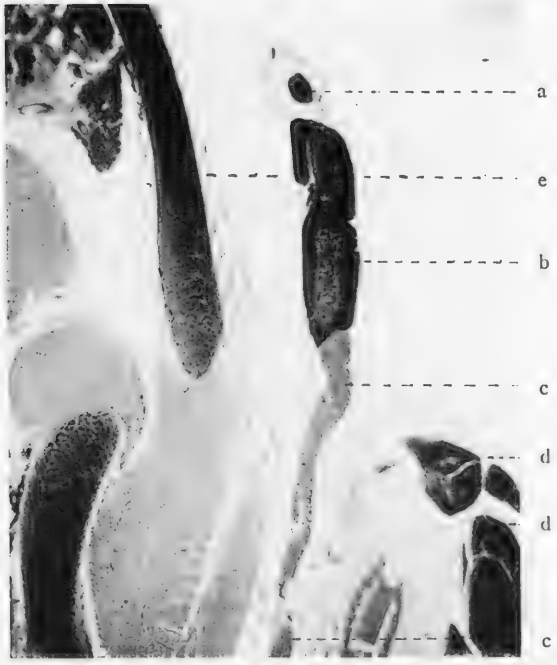

FIG. 2.

(Park: Extirpation of the Thymus in the Guinea Pig.)

FIG. 3.

FIG. 4.

(Park: Extirpation of the Thymus in the Guinea Pig.)

EXPERIMENTAL STUDIES ON THE RELATION OF THE PITUITARY BODY TO RENAL FUNCTION.

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INTRODUCTION.

In recent papers I have dealt with the clinical aspects of the relation between the pituitary body and the kidneys, especially with regard to the etiology and pathology of diabetes insipidus. I have come to the conclusion that the pituitary body, as shown by its extracts, exerts a constant, physiological influence on the functional activity of the kidneys in human beings. This action consists in a checking of the flow of urine,—an antidiuretic effect which is most marked when the diuresis is high. I have also reported a case of diabetes insipidus in which organotherapy with the posterior lobe of the pituitary body has been successfully carried out for a period of about 2 years.

This field for investigation is a comparatively new one, and the subject is of great importance not only from a physiological point of view, but from the promise it gives of an improved therapy. During the past year I have investigated this question experimentally.

HISTORICAL.

That there is some connection between the pituitary body and renal activity was first shown in 1901 by Magnus and Schäfer. They found a pronounced increase in the flow of urine in dogs, cats, monkeys, and rats, following the injections of pituitary extracts, and concluded that this influence was exerted directly on the epithelial elements of the kidneys. The same results were obtained by Schäfer and Herring in 1906, though their records show that the influence is far from constant.

Houghton and Merrill, in 1908, found that the increase in flow of urine in the dog lasted on an average of 19 minutes, and that the effect was dependent on the increase in the general blood pressure. A year later Halliburton, Candler,

and Sikes claimed that the extracts caused an increased output in cats, and in 1910 Ott and Scott came to the same conclusion.

Pentimalli and Quercia, in 1912, from experiments on the isolated kidney of the rabbit, showed that hypophysial extracts diminished the flow as well from the ureter as from the renal vein.

King and Stoland, in 1913, working on etherized dogs, were able to demonstrate an increase in the general blood pressure for 10 minutes, accompanied by swelling of the kidney with subsequent temporary increased flow of urine lasting for 20 minutes. They hold the view that the increased flow is due to renal vasodilatation. In the same year Gabriels, working on the isolated kidney of the dog, found increased flow without vasodilatation. On the other hand, Beco and Plumier, in 1913, noted a transient diminution of flow. In the same year Iscovesco maintained that a lipoid from the anterior lobe had a diuretic action both in rabbits and man.

In 1914, Römer reported that the flow was decreased in rabbits and cats, catheterized every hour.

Hoskins and Means, in 1912, concluded that the diuretic effect found in dogs under narcosis was not due to changes in blood pressure.

Herring, in 1913 and 1914, stated that the diuretic effect of the posterior lobe extracts could be demonstrated in all species of animals, and that extracts from the anterior lobe and the pars intermedia did not possess this power.

Cow, in 1914, found that pituitrin had a diuretic action of 15 minutes' duration in cats.

In 1916, von Meyenburg showed that the injection of extracts caused a decreased flow in rabbits of 8 to 10 hours' duration.

Shamoff, in 1916, found that electric stimulation of the superior cervical ganglion produced increased flow due to excitation of the pituitary body, but his results were inconstant.

The results obtained from observations in man are almost as contradictory.

De Cyon, in 1910, mentions that he had seen diuretic effects from feeding dry pituitary preparations.

Falta, Newburgh, and Nobel, in 1911, found increased diuresis a common result from subcutaneous injections of pituitrin.

Von den Velden, in 1913, was able to check the diuresis in two normal men by hypophysin, and in the same year Frey and Kumpiess confirmed this result by injections of pituglandol. 2 years later von Konschegg and Schuster obtained the same result in four normal subjects. Motzfeldt, in 1914, also found that injections of pituglandol checked the diuresis in a series of fifteen relatively healthy patients, while the anterior lobe extracts did not possess this property.

During the past 3 years a number of cases of diabetes insipidus have been reported in which pituitary extracts have checked diuresis to a considerable extent.

As will be seen, there has been great confusion concerning this question, and this is due chiefly to the fact that results of the experiments in the physiological laboratories have been too readily accepted by clinicians. As a matter of fact, every text-book dealing with this matter states as an ascertained fact that extracts from the posterior lobe are diuretics.

At this point it should be emphasized that the term diuresis is used with different meanings. Some authors use the word to indicate the total 24 hour amount of urine, without regard to the quantity, while others define the term as increase of the quantity of urine.

The confusion is due chiefly to the methods employed. Schäfer carried out his original experiments on anesthetized animals with the drop-recording method, and the observation time was generally very short. The urine was collected from a cannula inserted directly into the bladder. Under these circumstances it cannot be excluded that the increased number of drops may be due, at least partly, to the tonic action on the bladder, which is a constant effect of pituitary injections. There are, moreover, serious objections to operations on the bladder or ureter, as reflex action may interfere considerably with the normal conditions. Furthermore, as we do not know the influence of narcosis on the vegetative nervous system, work on anesthetized animals introduces an uncontrollable source of error.

Most of the subsequent observations have been made by Schäfer's original method, with the same errors and the same misinterpretation of the results. The results, however, have been notably inconstant in the reports of all. Schäfer and Herring, for instance, in 19 experiments on dogs, observed a diuretic effect in 12, and in 7 a diminution of flow. Moreover, most of the work has been carried out with intravenous injections which usually influence the blood pressure considerably.

Method.

The chief aim in finding a suitable method has been to avoid the sources of error mentioned above, and to work as closely as possible under physiological conditions. The work was started on dogs, but experience soon taught that rabbits were more suitable for this

purpose and the majority of the experiments, therefore, have been carried out on these animals.

Having made sure that the 24 hour amount of urine was diminished with three pituitrin injections given at 8 hour intervals, the half hourly output was followed. The animals were placed on the table on their backs and catheterized every half hour. Catheterization of male rabbits is easily done by a soft rubber catheter, and consequently the experiments were carried out on males exclusively.

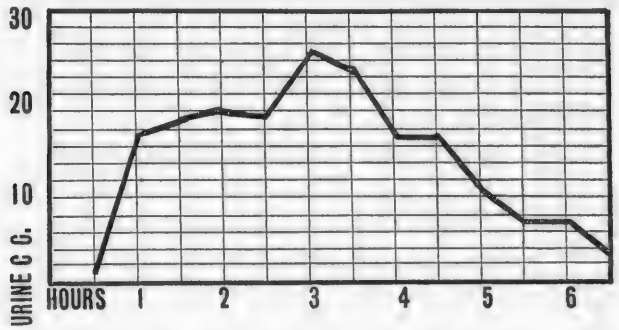
The experience of previous investigators has shown that the position on the back does not interfere with the normal flow of urine and the animals keep perfectly still without anesthesia. Psychic influences, therefore, can be ruled out.

In order to obtain a more marked reaction, I subsequently produced an artificial polyuria. For this purpose 150 cc. and later 200 cc. of water (about 37°C.) were introduced by stomach tube immediately before the animals were placed on the table. This amount is approximately the maximum which the stomach of a medium sized rabbit can hold. This method is much the best way of giving water, the subcutaneous or intravenous administration being less reliable and often unsatisfactory. Wherever the term "artificial polyuria" is used in the legends of the charts, it is to be understood that the polyuria is produced in the way described above.

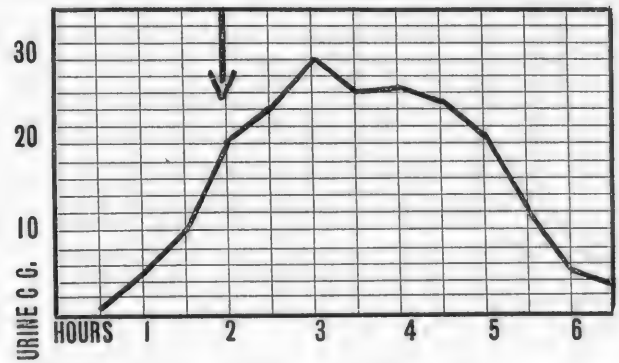
The degree of polyuria is dependent on many factors, particularly, however, on the water content of the tissues, and since this is controlled chiefly by the food, it is of importance to avoid a dry diet and feed the animals mainly on fresh vegetables. Rabbits have occasionally been used several times, as often as every other day, and this is of value because after introduction of water once or twice, the polyuria starts more readily and reaches higher degrees than on the first occasion. The half hourly amounts of urine, obtained in this way, have been plotted as curves, and the resultant polyuria has served as an excellent measure in testing the antidiuretic effect. Only in exceptional instances has the polyuria been delayed for several hours, and, when first started, spontaneous depressions of the output have never been observed.

The polyuria curve is not uniform, and the total amount of urine

returned is subject to great variation. Usually, however, the polyuria starts in 1 to 2 hours and is finished within 4 to 5 hours from the onset (Text-figs. 1 and 2). In estimating the antidiuretic effect, it is, therefore, the relative values which are of chief importance.



TEXT-FIG. 1. Rabbit, weight 2,100 gm. Artificial polyuria. (Water given at the beginning of the observation is indicated by zero.)



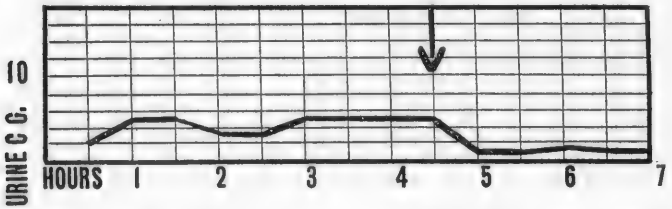
TEXT-FIG. 2. Rabbit, weight 1,500 gm. Artificial polyuria. The arrow indicates 0.7 per cent saline solution, 1 cc. intravenously (as control).

The pituitary extracts employed have been the usual commercial preparations: Pituitrin (Parke, Davis and Company), Pituitary Liquid (Armour and Company), Pituitary Extract (H. K. Mulford Company), Pituglandol (Hoffmann La Roche), Hypophysin (Farbwerke-Hoechst Company), and Extract Hypophysis (Schering and Glatz).

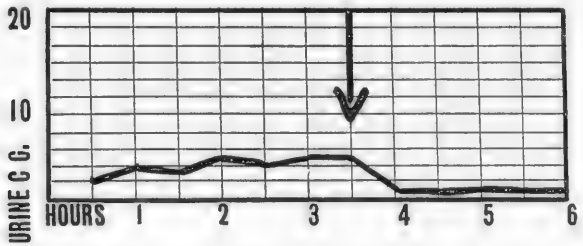
The majority of the experiments have been done without blood pressure registration, and oncometer determinations have not been employed.

Effect of Pituitary Extracts.

The normal half hourly output of urine in rabbits is subject to considerable variation and there is no dependable normal. But even under these circumstances the antidiuretic effect (Text-figs. 3 and 4) of hypophysial extracts is plainly shown (11 observations). It may



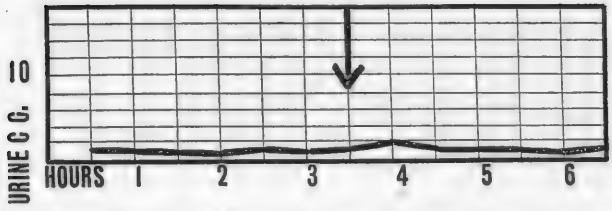
TEXT-FIG. 3. Rabbit, weight 1,400 gm. Normal output of urine. The arrow indicates pituitrin, 1 cc. subcutaneously.



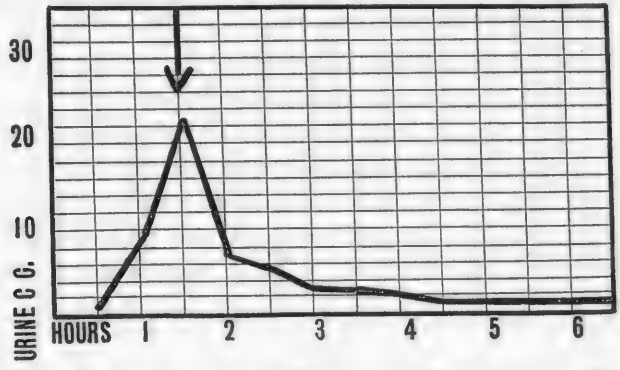
TEXT FIG. 4. Rabbit, weight 1,500 gm. Normal output of urine. The arrow indicates pituitrin, dilution 1: 10, 1 cc. intravenously.

be noted also that the subcutaneous and intravenous injections of the extracts act in the same way, and that when the output is very low (Text-fig. 5) there is no effect to be seen from the injection. This seems to indicate that there is a certain lower limit beyond which the output cannot be depressed. The effect of the extracts on artificial polyuria, however, is far more pronounced (Text-figs. 6 and 7).

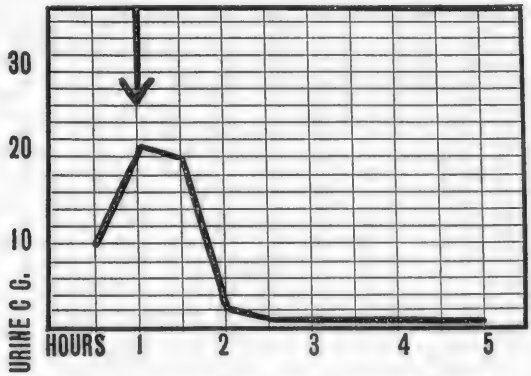
Pituitrin diluted 1: 50, subcutaneously injected in 1 cc. doses, evidently has power to check the output for approximately 5 to 6



TEXT-FIG. 5. Rabbit, weight 1,200 gm. Normal output of urine. The arrow indicates pituitrin, dilution 1: 10, 1 cc. subcutaneously.

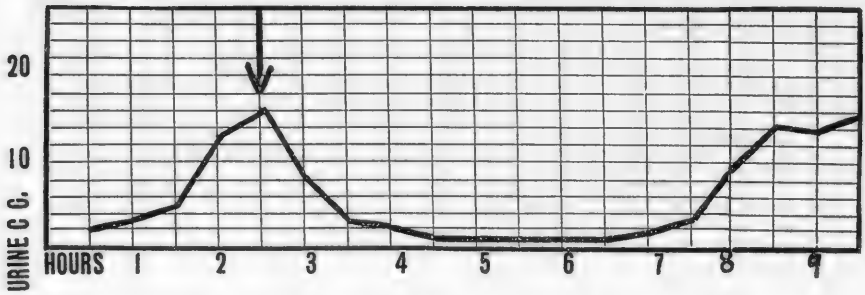


TEXT-FIG. 6. Rabbit, weight 2,000 gm. Artificial polyuria. The arrow indicates pituitary liquid, 1 cc. subcutaneously.

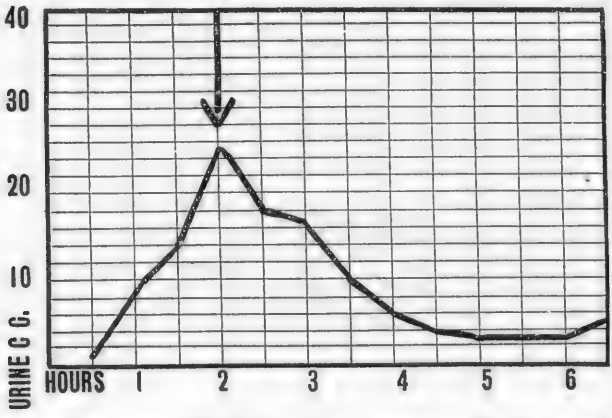


TEXT-FIG. 7. Rabbit, weight 2,400 gm. Artificial polyuria. The arrow indicates pituitary liquid, dilution 1: 10, 1 cc. subcutaneously.

hours (Text-fig. 8). Moreover, it is to be noted (Text-fig. 7) that the influence at times is little marked during the first 30 minutes, and generally reaches its maximum about 2 hours after the injection. This antidiuretic influence may be regarded as constant, for I have



TEXT-FIG. 8. Rabbit, weight 1,600 gm. Artificial polyuria. The arrow indicates pituitrin, dilution 1: 50, 1 cc. subcutaneously.



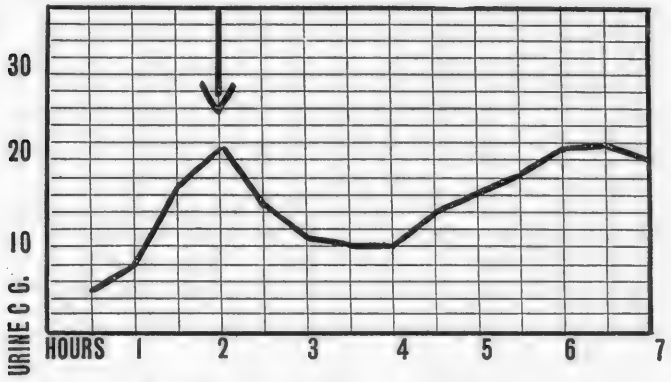
TEXT-FIG. 9. Rabbit, weight 1,400 gm. Artificial polyuria. The arrow indicates pituitrin "400%," 3 cc. by mouth.

seen no exceptions to this rule. The different pituitary extracts have all acted in the same way.

When the urinary output is depressed, the concentration changes in correspondence with the quantity excreted, from colorless to yellowish brown.

The preparations have not been compared as to limit of activity. Most of the work has been done with pituitrin, and the smallest active dose of this extract depends upon its method of administration.

Orally.—(9 observations.) Here there is a slow onset of the renal depression reaching its maximum in about 2 to 3 hours (Text-fig. 9[1]). The lower limit of activity for administration by mouth is apparently 1 cc. of pituitrin (Text-fig. 10).

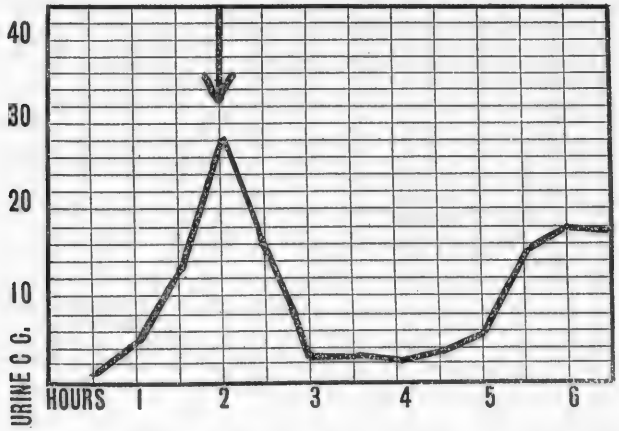


TEXT-FIG. 10. Rabbit, weight 1,500 gm. Artificial polyuria. The arrow indicates pituitrin, 1 cc. by mouth.

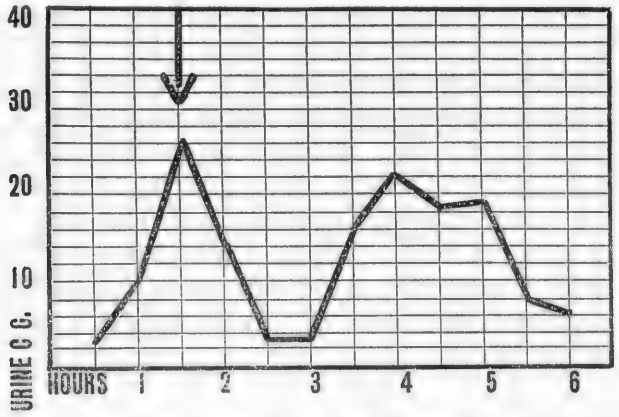
Subcutaneously.—(32 observations.) (Text-figs. 11, 12, and 13.) Here the onset is more abrupt, and with decreasing doses the duration of the effect is shorter. In small doses the effect is diminished also to the extent of the depression. These curves seem to indicate a limit of activity in dilution between 1:100,000 and 1:200,000, but the action is not constant beyond a dilution of 1:10,000. How this may be accounted for is unknown. On the other hand, the effect of large doses has been tried with the Parke, Davis preparation "400%." The action sets in rapidly, but even with this enormous dose, the output does not fall below 1 to 2 cc. in half an hour (Text-fig. 14). Apparently it is impossible to produce anuria in this way. The injections were well borne, except for cutaneous necrosis,

[1] Pituitrin "400%" is a special preparation (Parke, Davis and Company), which is four times the normal strength of pituitrin.

probably due to long lasting anemia of the skin, and the animals lived for a long time. Autopsy did not reveal pathological changes.



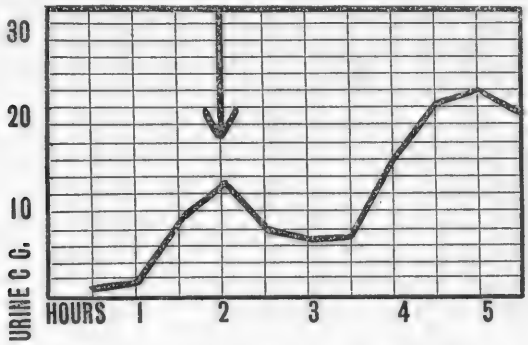
TEXT-FIG. 11. Rabbit, weight 1,600 gm. Artificial polyuria. The arrow indicates pituitrin, dilution 1: 800, 1 cc. subcutaneously.



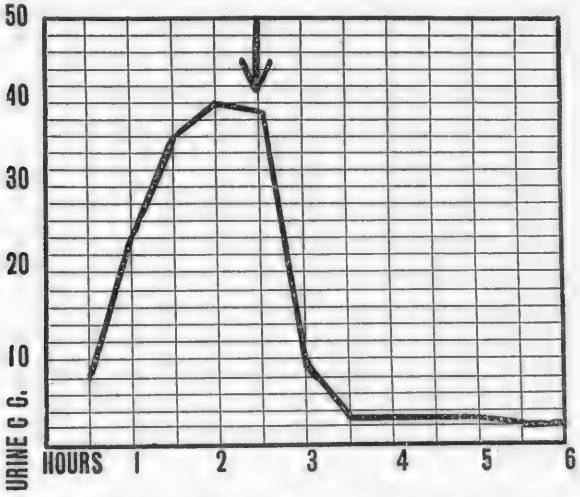
TEXT-FIG. 12. Rabbit, weight 1,800 gm. Artificial polyuria. The arrow indicates pituitrin, dilution 1: 5,000, 1 cc. subcutaneously.

Intravenously.—(8 observations.) This mode of administration shows the same result of decreasing doses as to duration and degree

of depression (Text-figs. 15 and 16). Evidently the limit by this method is beyond 1 cc., dilution 1: 500,000 (Text-fig. 16), which



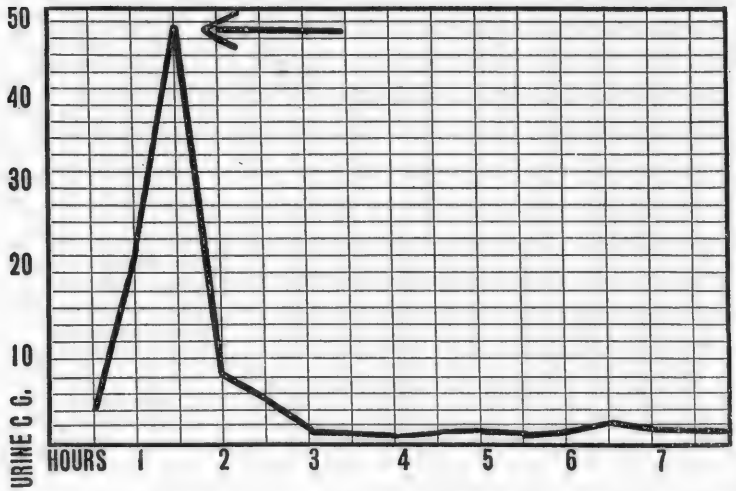
TEXT-FIG. 13. Rabbit, weight 2,200 gm. Artificial polyuria. The arrow indicates pituitrin, dilution 1: 100,000, 1 cc. subcutaneously.



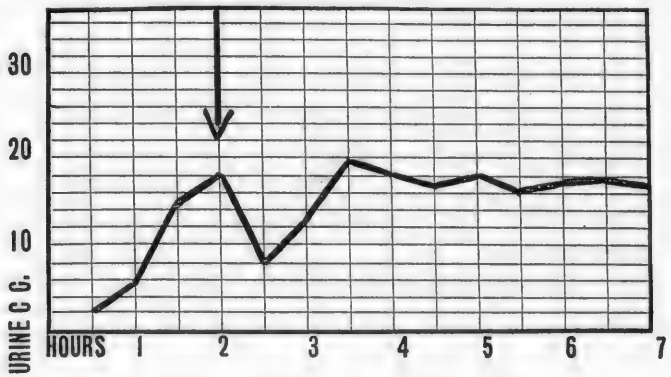
TEXT FIG. 14. Rabbit, weight 2,100 gm. Artificial polyuria. The arrow indicates pituitrin "400%," 12 cc. subcutaneously.

might possibly be of value as a biological test for the presence of pituitary secretion. My observations, however, are too few to warrant conclusions as to its reliability for this purpose. The effect

sets in more quickly, but in some instances there has been a latent period even by intravenous injections.



TEXT-FIG. 15. Rabbit, weight 2,800 gm. Artificial polyuria. The arrow indicates pituitrin, 2 cc. intravenously.



TEXT-FIG. 16. Rabbit, weight 1,500 gm. Artificial polyuria. The arrow indicates pituitrin, dilution 1: 500,000, 1 cc. intravenously.

As already mentioned, dogs are not suitable for experiments of this kind on account of the high concentration of their urine. I have,

however, made two observations on a dog in which polyuria had developed after partial pancreatectomy.[2]

Observation 1.—Dog; weight 13.2 kg. October, 1915. Partial pancreatectomy. Lowered carbohydrate tolerance. Sugar-free on meat diet. Diuresis averaged 200 to 300 cc. April, 1916, diuresis averaged 500 to 700 cc. (no sugar).

Pituitrin 1 Cc. Five Times Subcutaneously.

	Diuresis.	
	cc.	*Sp. gr.*
Apr. 27	600	1,028
" 28	460	1,025
" 29	500	1,032
" 30	620	1,035
May 1	600	1,040
" 2	650	1,028
" 3	750	1,032
" 4	400	1,034
" 5	200	1,047
" 6	700	1,030

Observation 2.—The same dog.

Pituitrin 1 Cc. Three Times Subcutaneously.

	Diuresis.	
	cc.	*Sp. gr.*
May 9	850	1,015
" 10	900	1,013
" 11	250	1,047
" 12	600	1,030
" 13	770	1,017
" 14	600	1,016
" 15	520	1,028

Is This Effect Dependent on Changes in General Blood Pressure?

A priori this seems unlikely, as it is agreed by most investigators that a subcutaneous injection of pituitrin exerts a slight influence or none on the blood pressure, and it would require a pronounced drop to account for the antidiuretic effect. Three observations in which the pressure has been registered for 3 hours after subcutaneous injection with pituitrin, have shown no change in pressure, while the output has been checked.

[2] I am indebted to Dr. J. Homans for the opportunity of making these observations.

To Which Part of the Pituitary Body Is This Action Due?

Watery extracts have been prepared from different parts of the same lot of bovine glands, made up in the same way as is pituitrin.

Anterior lobe extract (5 observations) gave a definite antidiuretic reaction in a dose of 1 cc., while 1 cc. of a dilution of 1: 50 gave negative results.

The extracts from the pars intermedia (7 observations) and the posterior lobe (10 observations), however, had a marked antidiuretic influence in a dilution of 1:40,000 (1 cc. injected). The intermedia extract showed this effect most constantly, while the posterior lobe extract failed in some instances long before the dilution 1: 40,000 was reached. The dilutions were not carried beyond 1:40,000, and subcutaneous injections were used exclusively.

Two other extracts from the anterior lobe (Parke, Davis and Company) also showed negative results when 1 cc. of a dilution of 1:50 was injected. These anterior lobe extracts have also produced a marked response on the guinea pig uterus (Dale apparatus).

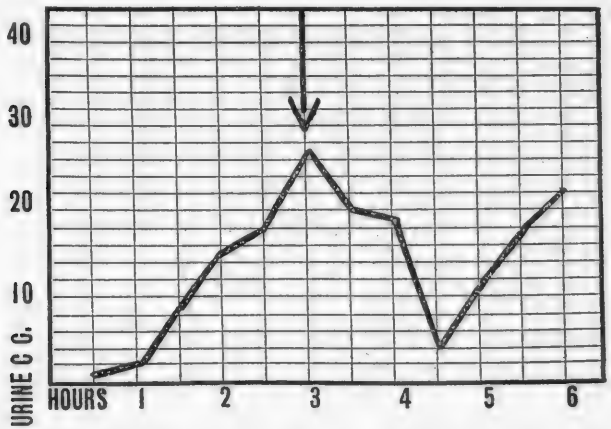
A special anterior lobe preparation, tethelin, which Robertson claims to be the growth-controlling principle of that lobe, has further been tested (4 observations). 1 cc. of a 5 per cent solution of this substance had a marked antidiuretic effect, and the limit of activity is between dilutions of 1: 10 and 1: 100.

It would thus appear justifiable to assume that the anterior lobe is relatively weak in antidiuretic action, while this power seems to be equally manifested by the pars intermedia and by the posterior lobe. These results are in agreement with clinical experience in as far as anterior lobe extracts are unable to check the output in diabetes insipidus.

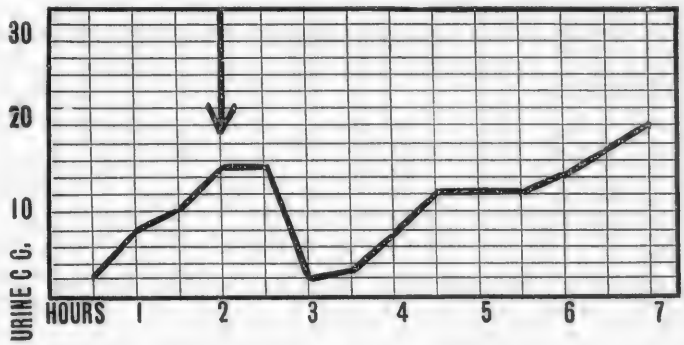
Active Principles of the Hypophysis.

In spite of the large amount of work done upon this subject, especially in the chemical laboratories of some of the leading drug firms, our knowledge is comparatively limited. Many compounds of doubtful specificity have been isolated, but although the term "active principle" is not yet justifiable, some of the bases isolated may be regarded at least in some way as functional equivalents. One of these compounds, β-imidazolylethylamine, is already used for the standardization of pituitary extracts.

Owing to the European war it has been very difficult to obtain these materials, and I have been able to secure small samples only of some of the compounds (Hoffmann La Roche).



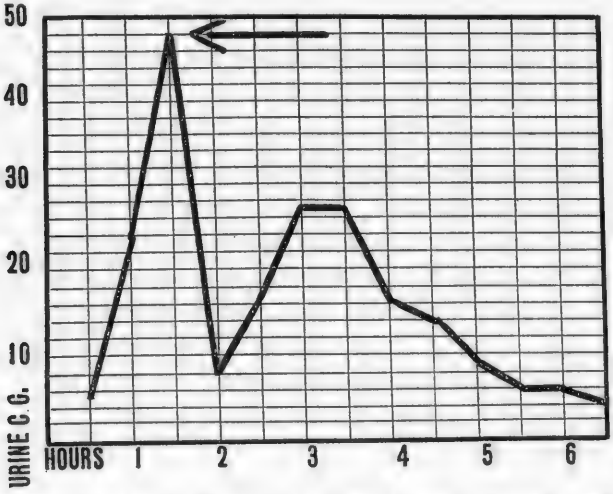
TEXT-FIG. 17. Rabbit, weight 1,500 gm. Artificial polyuria. The arrow indicates β-imidazolylethylamine (Hoffmann La Roche), 20 mg. by mouth.



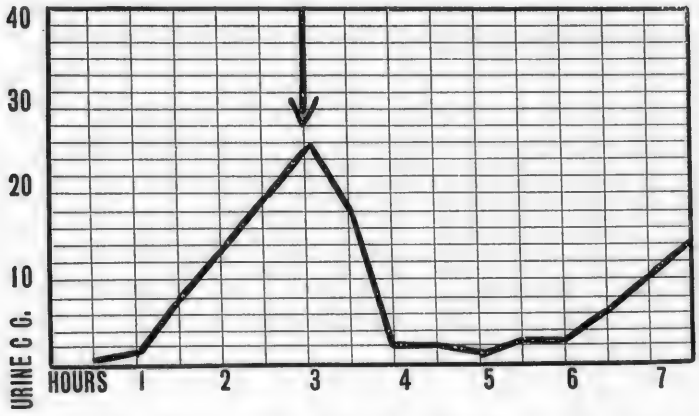
TEXT-FIG. 18. Rabbit, weight 1,700 gm. Artificial polyuria. The arrow indicates β-imidazolylethylamine, 1 mg. subcutaneously.

β-Imidazolylethylamine. Orally.—(5 observations.) For 20 mg., the curves (Text-fig. 17) are in perfect agreement with the pituitary curves. 10 mg. had no such effect, and when exposed to air and light for 24 hours the solution had become inactive.

Subcutaneously.—(6 observations.) The curve for 1 mg. showed a positive result (Text-fig. 18), while 0.4 mg. only gave a slight antidiuretic response.



TEXT-FIG. 19. Rabbit, weight 2,000 gm. Artificial polyuria. The arrow indicates β-imidazolylethylamine, 1.5 mg. intravenously.

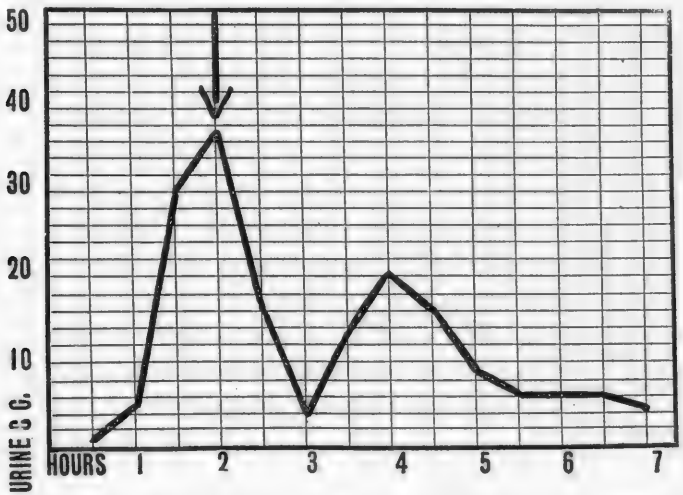


TEXT-FIG. 20. Rabbit, weight 1,500 gm. Artificial polyuria. The arrow indicates *p*-oxyphenylethylamine (Hoffmann La Roche), 10 mg. subcutaneously.

Intravenously.—(2 observations.) Strongly positive results were obtained for 1.5 mg. (Text-fig. 19).

p-Oxyphenylethylamine.—(1 observation.) Strongly positive results were obtained with 10 mg. subcutaneously injected (Text-fig. 20).

Experiments with the mother substances of these amines, histidine (1 observation) and phenylethylamine (1 observation), in 10 mg. doses, gave negative results. In this connection it will be of interest to see the effect of Secale (5 observations), the drug from which Barger and Dale first isolated β-imidazolylethylamine. A prepara-



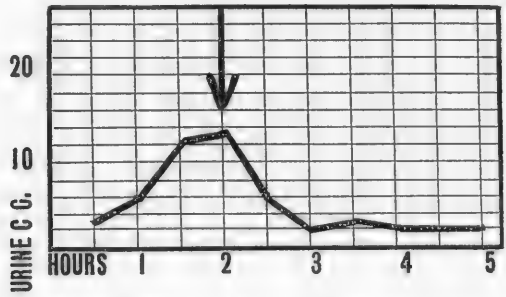
TEXT-FIG. 21. Rabbit, weight 2,000 gm. Artificial polyuria. The arrow indicates secacornin (Hoffmann La Roche), dilution 1: 10, 1 cc. subcutaneously.

tion from this drug gave positive results in a 1: 10 dilution (Text-fig. 21), while the reaction was almost negative in a dilution of 1: 50. This finding will naturally imply that the sympathetic nervous system or the blood vessels were involved in the action. Moreover, it is of interest because Secale is one of the drugs recommended in diabetes insipidus.

These results are suggestive, as the compounds belong to the series of amines which Barger and Dale have named "sympathomimetic

amines" on account of their general stimulating effect on the sympathetic nervous system. Unfortunately, it is at present impossible to procure in America other members of this highly interesting chemical group. Their antidiuretic action has not previously been demonstrated, but Dale and Laidlaw observed a decrease in the volume of the kidney following injection with β-imidazolylethylamine, which they believed due to active renal vasoconstriction. They found no effect on the secretion of urine,—probably on account of the short observation time.

Barbour and Quagliariello have been able to show that β-imidazolylethylamine constricts isolated arteries.



TEXT-FIG. 22. Rabbit, weight 1,500 gm. 0.7 per cent sodium chloride solution, 200 cc. subcutaneously. The arrow indicates pituitrin, 1 cc. subcutaneously.

Parenteral Water Administration.

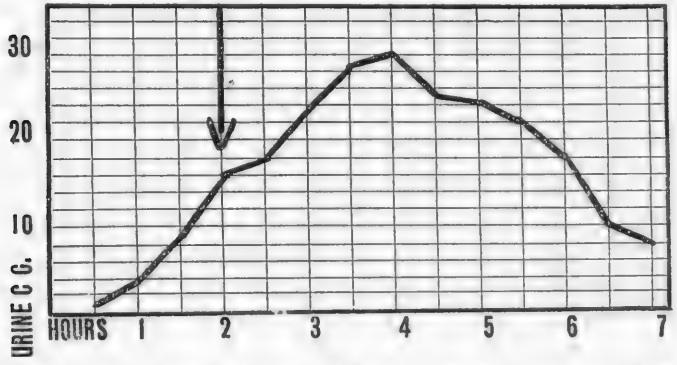
In order to rule out the remote possibility that the antidiuretic action might be due to influence on the intestinal absorption, some experiments have been performed in which polyuria was produced by saline solution, administered subcutaneously (3 observations). The pituitary extracts acted in the usual way (Text-fig. 22).

Influence of the Nervous System.

The marked influence of the nervous system upon the kidneys has been considered chiefly of vasomotor nature, the existence of true secretory nerves being as yet unproved.

Vagi.—(10 observations.) Opinions have differed considerably as to the influence of the vagi. Increase or decrease in output of urine has been described as the result both of stimulation and of division of the nerve. Some investigators, however, have been unable to demonstrate any influence.

The vagi were severed in the neck before beginning the experiments. This technique, however, proved unsatisfactory as the onset of polyuria was either delayed or did not occur, and I have reason to believe that this was due to gastric paralysis whereby the ingested water was not moved on. It was necessary, therefore, to postpone the division of the nerves until the water had left the stomach and polyuria had set in. The operations were finished in 5 to 10 minutes and performed under ether narcosis. This procedure (Text-fig. 23) does not interfere with the normal course of the artificial polyuria, and gives a good test-object. The output, however, is checked in the usual way by pituitary injections (Text-fig. 24).

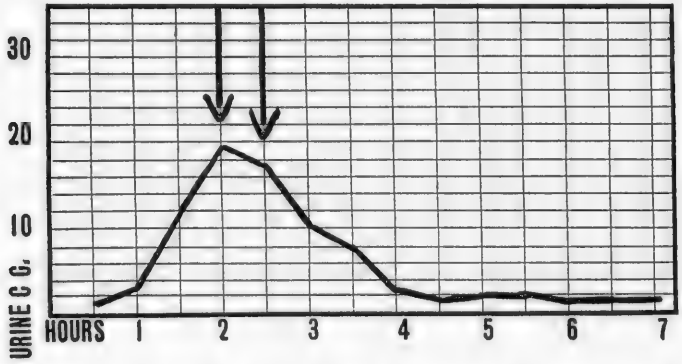


TEXT-FIG. 23. Rabbit, weight 2,000 gm. Artificial polyuria. The arrow indicates the division of the nervi vagi in the neck.

Splanchnic Nerves.—(8 observations.) Most investigators agree that stimulation tends to diminish the flow of urine. Division of the nerve is supposed to bring about polyuria in dogs but not in rabbits, though Jungmann and Meyer have observed polyuria of short duration also in these animals. The influence of the splanchnics is generally supposed to be dependent on vasomotor changes.

Section of both splanchnic nerves did not suit my purpose, as polyuria was not readily produced. This may be accounted for partly by the low blood pressure, which is apt to persist after the operation, and partly by the tendency of the animals to develop diarrhea upon water ingestion. Consequently only one splanchnic was cut and the kidney of the other side removed, this procedure being considered safe as several earlier investigators agree that the activity exerted by the splanchnic nerve on the kidney is strictly unilateral.

Splanchnicotomy in rabbits is a difficult operation and this undoubtedly accounts for the varying results. The nerve was exposed

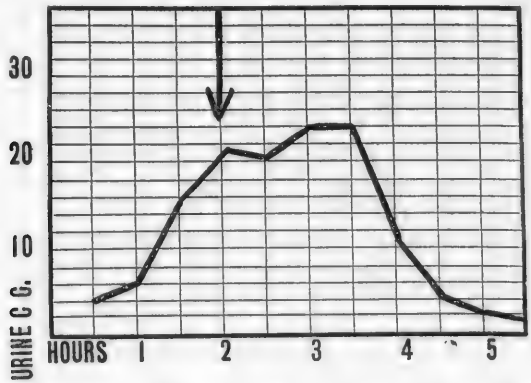


TEXT-FIG. 24. Rabbit, weight 1,400 gm. Artificial polyuria. The first arrow indicates the division of the nervi vagi. The second arrow indicates pituitary liquid, 1 cc. subcutaneously.

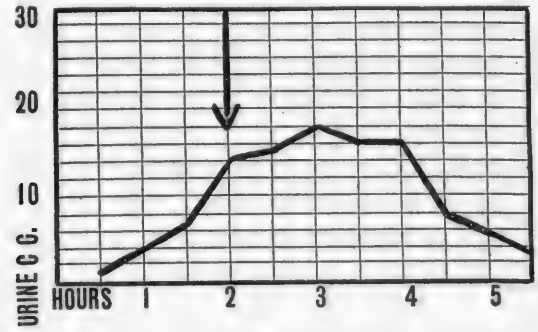
from the back in the retroperitoneal space and divided about 1 cm. above the adrenal, the procedure being performed under ether anesthesia. The nerve is small and the field narrow, and it is difficult, therefore, to be certain that all fibers have been cut. The observations were made 1 to 8 days after operation.

Some of the observations showed the usual response upon pituitary injection, but in others apparently no influence could be demonstrated (Text-figs. 25 and 26). Since, in my experience, 1 cc. of pituitary extract has never failed to check polyuria, I believe it justifiable, despite the limited number of experiments, to infer that the splanchnic nerve is the pathway for the normal antidiuretic action of

the pituitary extracts. If the curves do not justify the conclusion that the influence is entirely absent, it must at least be granted that the reaction is considerably delayed.



TEXT-FIG. 25. Rabbit, weight 1,600 gm. Division of the left splanchnic nerve and removal of the right kidney, 5 days before the observation. Artificial polyuria. The arrow indicates pituitrin, 1 cc. subcutaneously.

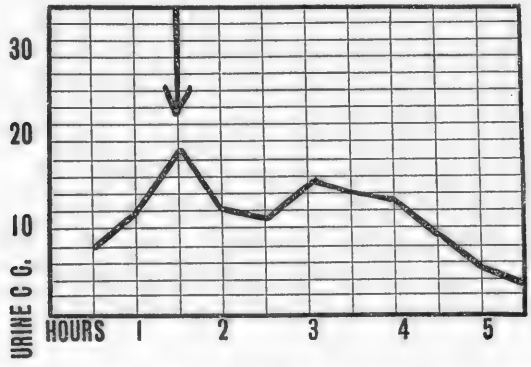


TEXT-FIG. 26. Rabbit, weight 2,000 gm. Division of the left splanchnic nerve and removal of the right kidney, 1 day before the observation. Artificial polyuria. The arrow indicates pituitrin, 1 cc. subcutaneously.

Renal Nerves.—(6 observations.) The kidneys of the rabbits were exposed transperitoneally, under anesthesia, through a median abdominal incision and the vessels carefully dissected near the hilus; the visible nerves were cut, and the outer layer of the artery partly

removed. As nerve fibers must still remain in the wall of the artery, it is impossible in this way to exclude nervous influence entirely, but approximately 90 per cent of the fibers are supposed to be divided. The observations were made 2 to 5 days after operation. Some of the animals gave the usual response to pituitrin while others acted in an unusual manner, the differences being possibly referable to variation in the number of functionally intact nerve fibers. As regards both degree and duration, some of the experiments show that the reaction is less pronounced than usual (Text-fig. 27).

The investigations of von Frankl-Hochwart and Fröhlich indicate that pituitrin acts as a stimulus to certain nerves belonging to the



TEXT-FIG. 27. Rabbit, weight 2,400 gm. Division of the renal nerves, 8 days before the observation. Artificial polyuria. The arrow indicates pituitrin, 1 cc. subcutaneously.

sympathetic as well as to the parasympathetic system, while the influence, according to Dale, is exerted directly on the involuntary muscles. As to the kidneys, however, this point has not been tested.

Is the Antidiuretic Influence Caused by Vasomotor Changes?

The nervous system has, as has been shown, a definite relation to this effect of the pituitary extracts, and the influence may be of vasomotor or secretory origin. As the existence of true secretory renal nerves remains doubtful, a foundation for discussion of this question is lacking. The general vasoconstrictor influence of pituitary ex-

tracts is, on the other hand, well known, but as to the renal vessels there is much confusion, apparently due to the methods employed.

Schäfer and his coworkers noted dilatation of the renal vessels, and Campbell, working on isolated renal vessels, confirmed this finding, while Dale found constriction of the isolated kidney. Pentimalli and Quercia, in perfusion experiments, observed decreased flow from the renal vein. Pal was able to demonstrate that strips from the outer layer relaxed, while the central parts contracted.

In dealing with these problems emphasis must be placed on the existence of peripheral vasomotor centers, the existence of which, in renal vessels, may be regarded as well established. It is, therefore, not justifiable to rule out nervous influence even after section of the nerves. It is possible, furthermore, that antidiuretic influence may be produced while the pituitary extracts are, at the same time, working on the nervous system and directly on the smooth muscles of the vessels.

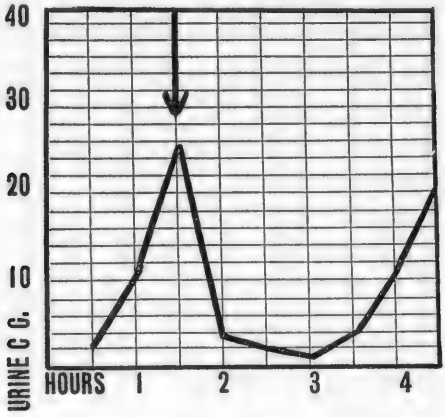
The vasoconstriction, which I believe is the chief cause of the checked output of urine, may be theoretically induced by stimulation of the vasoconstrictor or paralysis of the vasodilator nerves, but the assumption of an active vasoconstriction is the most likely explanation. With this assumption, the short antidiuretic response after dissection of the renal nerves may be accounted for by the fact that a part of the renal nerves between aorta and hilus is intact, and also by the remaining fibers in the wall of the vessels. Clinically, diabetes insipidus has for many years been considered to be due to vasomotor disturbances.

Pharmacological Studies.

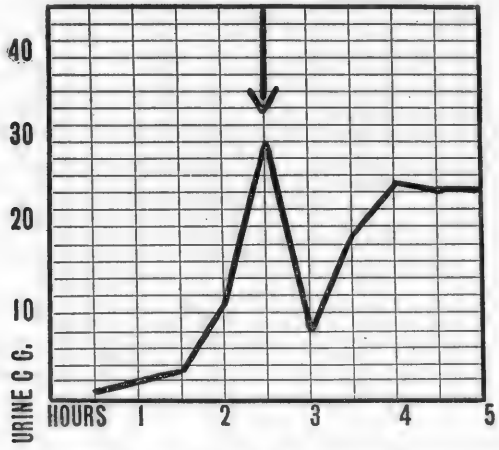
Nicotine.—(21 observations.) The influence of this drug shows a close similarity to the effect of the pituitary extracts (Text-fig. 28). The lower limit for the antidiuretic action of nicotine, which influence has not previously been demonstrated, is between 0.1 and 0.05 mg. intravenously injected into rabbits of medium size (Text-fig. 29). Subcutaneous injections with doses up to 30 mg. did not show such an effect. Two observations with blood pressure registration did not show changes in pressure during the time the output was checked.

Since the work of Langley and Dickinson, there has been a general

agreement that small intravenous doses of nicotine stimulate the sympathetic nervous system, and it therefore appears justifiable to



TEXT-FIG. 28. Rabbit, weight 2,900 gm. Artificial polyuria. The arrow indicates nicotine, 5 mg. intravenously.



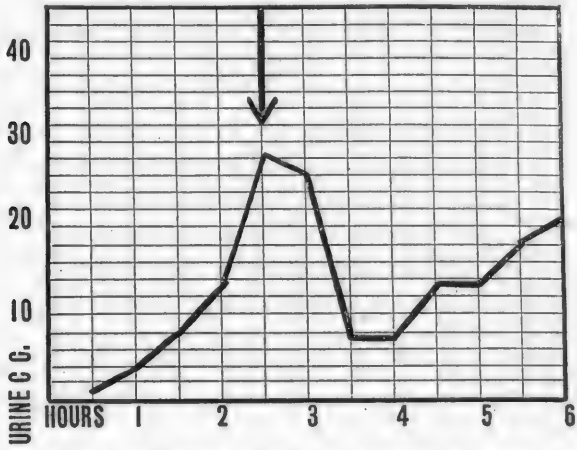
TEXT-FIG. 29. Rabbit, weight 1,500 gm. Artificial polyuria. The arrow indicates nicotine, 0.1 mg. intravenously.

refer the antidiuretic effect to such a stimulation. Judging, furthermore, from Langley and Dickinson's observation that nicotine,

painted on the renal plexus, produced constriction with subsequent dilatation of the renal vessels, it would seem that the antidiuretic power is exercised chiefly by means of these vessels.

I have not been able to block the sympathetic nervous system of the rabbit, as this requires large doses, 7 to 10 mg. per kilo intravenously being given as the smallest effective dose, and these are close to the fatal doses.

Caffeine.—(5 observations.) Subcutaneous injections with 0.15 gm. did not influence polyuria, while 0.20 and 0.25 gm. produced an inhibition of only 2 to 3 hours' duration (Text-fig. 30). This is prob-



TEXT-FIG. 30. Rabbit, weight 1,700 gm. Artificial polyuria. The arrow indicates caffeine, 0.25 gm. subcutaneously.

ably due to general vascular constriction, including the renal arteries, although toxic influence with lowered blood pressure could not be eliminated.

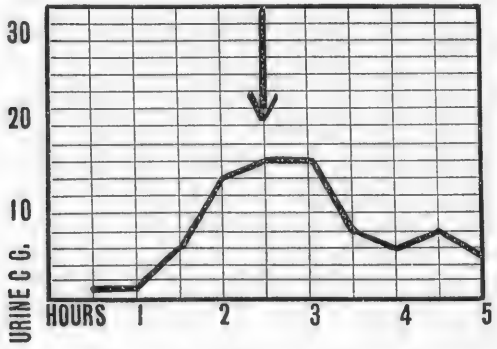
Strychnine.—(10 observations.) Subcutaneous injections with sulphate of strychnine, often in convulsive doses, from 0.06 to 0.54 mg., did not inhibit polyuria in rabbits weighing 1.5 to 2 kilos.

Strychnine is among the drugs usually recommended in diabetes insipidus.

Morphine.—(4 observations.) Subcutaneous injections with sul-

phate of morphine (8 to 65 mg. in rabbits weighing 1,500 to 1,900 gm.) did not show definite influence on the polyuria curves.

Chloral and Paraldehyde.—A series of experiments have been performed in which simultaneously with water ingestion chloral (10 observations) or paraldehyde (6 observations) has been administered orally. The doses have been chosen in accordance with what is given in the text-books as sufficient to paralyze the vasomotor nerves,—0.6 gm. of chloral per kilo, or 3 gm. of paraldehyde for a medium sized rabbit. These are the doses which are necessary to show the diuretic effect of caffeine, when permitted to act for 2 hours.



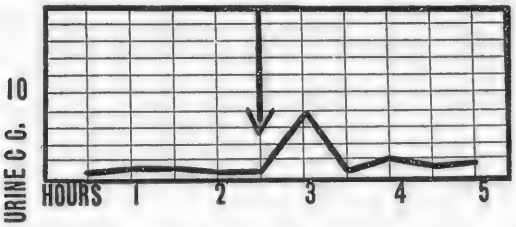
TEXT-FIG. 31. Rabbit, weight 1,300 gm. Artificial polyuria. Chloral 0.8 gm. by mouth. The arrow indicates pituitrin, 1 cc. subcutaneously.

I have been able to confirm the findings of earlier investigators that water diuresis is less readily produced under anesthesia, which fact is probably due to the low blood pressure. There are, however, serious objections against this method of blocking the vasomotor effect. The antidiuretic influence was very little changed under these circumstances (Text-fig. 31), except that the output in some instances did not go so low as after injection in normal animals. These observations disclosed another interesting fact; namely, that an inconstant initial diuretic effect occurred during the first 30 minutes (Text-fig. 32). This is no doubt the effect which has been found by Schäfer and most of the investigators who have worked with his method on anesthetized animals. This view may further be sup-

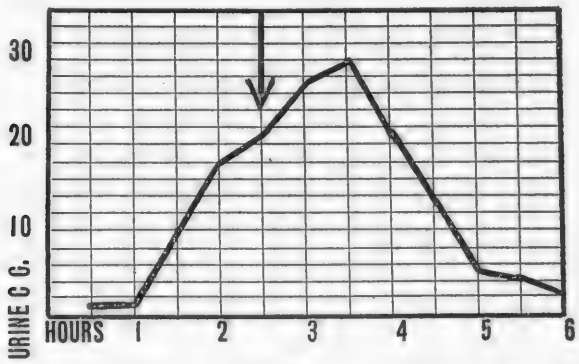
ported by Cow's general experience that when experimenting on unanesthetized animals the diuretic action of a substance may frequently be masked.

Influence of Other Ductless Glands.

Adrenal Medulla.—(28 observations.) Opinions have differed concerning the influence of adrenalin (Parke, Davis and Company)



TEXT-FIG. 32. Rabbit, weight 1,500 gm. Water, 20 cc. Chloral 0.9 gm. by mouth. The arrow indicates pituitrin, 1 cc. subcutaneously.

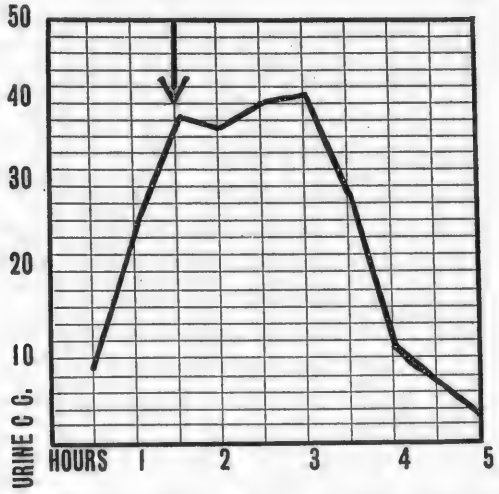


TEXT-FIG. 33. Rabbit, weight 1,800 gm. Artificial polyuria. The arrow indicates adrenalin, 2 mg. subcutaneously.

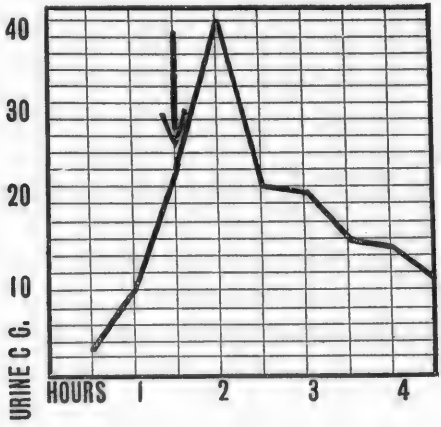
on diuresis. Most writers, however, agree that the general effect is a short, primary decrease and secondary increase in flow of urine. Judging from the knowledge that adrenalin has a constricting action also on the renal vessels and that pituitary extracts stimulate the adrenals to increased secretion, it would *a priori* appear reasonable to assume a cooperation between these glands as to antidiuretic effect.

Subcutaneously.—The polyuria is uninfluenced (Text-fig. 33).

Intravenously.—The appearance of the polyuria curve is not changed (Text-fig. 34).

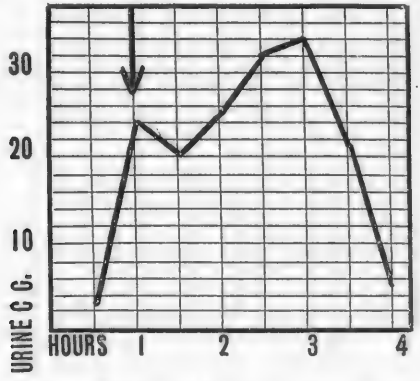


TEXT-FIG. 34. Rabbit, weight 1,700 gm. Artificial polyuria. The arrow indicates adrenalin, 0.01 gm. intravenously.



TEXT-FIG. 35. Rabbit, weight 3,000 gm. Artificial polyuria. The arrow indicates adrenalin, 0.05 mg. intravenously, every 3rd minute for 30 minutes.

Intravenously at Short Intervals.—As the effect of an intravenous injection with adrenalin is only of short duration, the drug has in some instances been administered intravenously every 3rd minute for about half an hour. In some instances there seems to be an increase in the output (Text-fig. 35), while other observations have shown a slight decrease (Text-fig. 36). An inhibition, however, corresponding to the effect of pituitary extracts has not been observed. Doses ranging from 0.001 to 2 mg. have been injected, and many of the animals have shown the usual signs of adrenalin intoxication at autopsy.



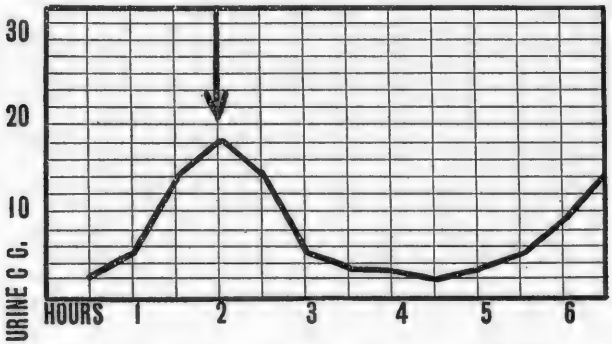
TEXT-FIG. 36. Rabbit, weight 2,900 gm. Artificial polyuria. The arrow indicates adrenalin, 0.05 mg. intravenously, every 3rd minute for 24 minutes.

In order to try the influence of other ductless glands, watery extracts have been prepared (Parke, Davis and Company) from different glands, made up in the same way as is pituitrin.

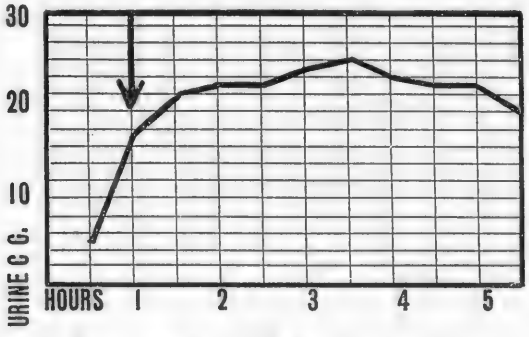
Adrenal Cortex.—(11 observations.) This showed a marked antidiuretic response, when administered subcutaneously in 1 and 2 cc. doses (Text-fig. 37), but the result was negative in a dilution of 1: 10, and extract containing medulla as well as cortex did not possess this power in 1 cc. doses. The antidiuretic action of the adrenal cortex is in full harmony with the therapeutic results which Belfield obtained in diabetes insipidus by feeding the cortex. It is not likely, however, that the adrenals are concerned in the etiology of diabetes insipidus, judging from the fact that polyuria is not seen either in Addison's disease or after experimental removal of the adrenals.

Pineal Gland.—(6 observations.) Subcutaneous (Text-fig. 38) and intravenous injections both gave negative results.

There are a few cases of diabetes insipidus on record in which tumors of the pineal gland are held responsible for the polyuria (von



TEXT-FIG. 37. Rabbit, weight 1,800 gm. Artificial polyuria. The arrow indicates adrenal cortex extract, 2 cc. subcutaneously.



TEXT-FIG. 38. Rabbit, weight 1,800 gm. Artificial polyuria. The arrow indicates pineal extract, 1 cc. subcutaneously.

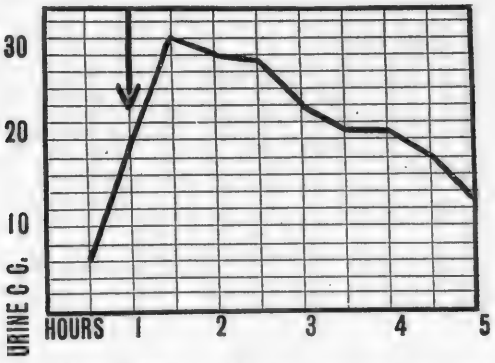
Gierke), but these cases may easily be referred to secondary influence on the pituitary body due to hydrocephalus.

Thyroid.—(6 observations.) Both subcutaneous (Text-fig. 39) and intravenous injections were negative.

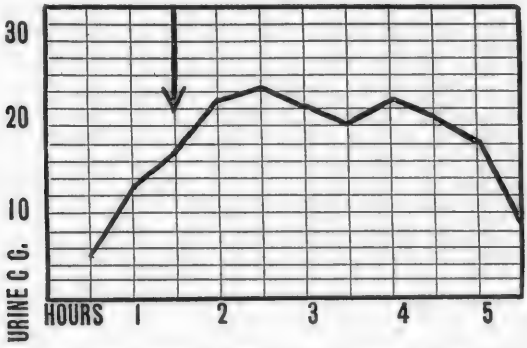
Thymus.—(7 observations.) The results were negative (Text-fig. 40).

Corpora lutea.—(5 observations.) These results were also negative (Text-fig. 41).

Pancreas.—(8 observations.) Injections gave negative results (Text-fig. 42) and no explanation is thus found for the polyuria com-



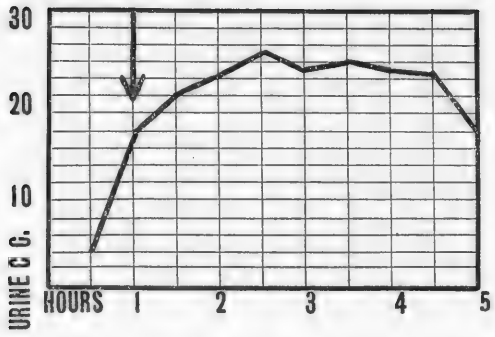
TEXT-FIG. 39. Rabbit, weight 1,900 gm. Artificial polyuria. The arrow indicates thyroid extract, 2 cc. subcutaneously.



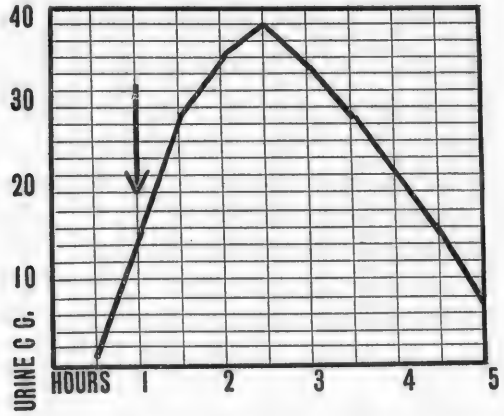
TEXT-FIG. 40. Rabbit, weight 2,600 gm. Artificial polyuria. The arrow indicates thymus extract, 1 cc. intravenously.

monly seen in dogs after partial pancreatectomy, or for the polyuria in diabetic patients.

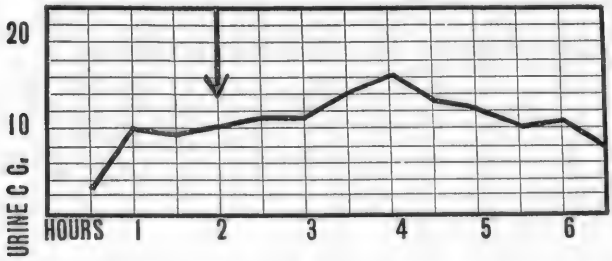
Ott and Scott have seen a diuretic effect from different ductless gland extracts, but the antidiuretic effect has not previously been tested.



TEXT-FIG. 41. Rabbit, weight 1,200 gm. Artificial polyuria. The arrow indicates corpora lutea extract, 1 cc. subcutaneously.



TEXT-FIG. 42. Rabbit, weight 2,100 gm. Artificial polyuria. The arrow indicates pancreas extract, 1 cc. intravenously.



TEXT-FIG. 43. Rabbit, weight 1,700 gm. 10 per cent sodium chloride solution, 40 cc. by mouth. The arrow indicates pituitrin, 1 cc. subcutaneously.

Influence on the Salt Diuresis.

Polyuria was produced for this purpose by ingestion by mouth of 40 cc. of a 10 per cent solution of sodium chloride (16 observations). The usual effective dose of pituitrin did not influence polyuria produced in this way (Text-fig. 43), or the influence was only slight. These results tend to demonstrate the fundamental differences between water diuresis and salt diuresis, and they show further the importance of a salt-poor diet in organotherapy of diabetes insipidus.

SUMMARY.

1. The inconstant results of past observations on the relation of pituitary extracts to renal activity have been due chiefly to unsuitable methods.

2. A standard curve of artificially induced polyuria may be plotted for rabbits, giving 200 cc. of water by mouth.

3. Extracts of the pars intermedia and posterior lobe of the hypophysis, given by mouth, subcutaneously, or intravenously, are able definitely to check polyuria thus induced. Extracts of the anterior lobe show a similar effect, but only to a slight degree.

4. This antidiuretic effect is constant, and is independent of (*a*) changes in blood pressure, (*b*) intestinal absorption, and (*c*) the vagi. The effect is apparently prevented or delayed by division of the splanchnics, and is diminished by division of the renal nerves near the hilus.

5. A similar antidiuretic property is possessed: (*a*) by β-imidazolylethylamine, (*b*) by *p*-oxyphenylethylamine, (*c*) by a preparation from *Secale cornutum*, (*d*) by small doses of nicotine, (*e*) by large doses of caffeine, and (*f*) by extracts of the adrenal cortex.

6. No effect on the polyuria is produced: (*a*) by strychnine, (*b*) by morphine, (*c*) by adrenalin, or by extracts of (*d*) thyroid, (*e*) thymus, (*f*) pineal, (*g*) pancreas, or (*h*) corpora lutea.

7. In animals under chloral or paraldehyde anesthesia a short and inconstant initial increase in flow of urine is seen.

8. The antidiuretic effect is absent or only slightly marked in checking the so called salt diuresis.

CONCLUSIONS.

These facts tend to suggest that the antidiuretic action exerted by pituitary extracts on rabbits is caused by stimulation of the sympathetic nervous system and that the renal vasomotor system in this respect is of chief importance.

Clinically these conceptions bring the polyurias related to disorders of the nervous system and the polyurias of pituitary origin in closer contact.

I wish to acknowledge the assistance of Dr. S. C. Harvey and Dr. W. C. Quinby in some of the operative procedures, the cooperation of Dr. J. Homans and Dr. T. Brailsford Robertson, and the courtesy of Hoffmann La Roche Company. I am indebted to Dr. Harvey Cushing for the privilege of carrying out this work in his laboratory under his direction.

I also wish to express special thanks to Dr. E. M. Houghton and Dr. C. P. McCord, of the biological laboratory of Parke, Davis and Company, for their help.

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THE PHAGOCYTIC POWER OF CONNECTIVE TISSUE CELLS.

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PLATES 22 AND 23.

(Received for publication, October 25, 1916.)

Suspensions of individual, living cells from the fixed tissues can be obtained by digesting with trypsin the clot of proliferating tissue cultures.[1] Certain of the cells thus freed, especially those of connective tissue and the sarcomata, will survive in Locke's solution for many hours, and proliferate when reimplanted in plasma. The method has made possible direct tests of the phagocytic power of fibroblasts.

In connective tissue *in vivo* phagocytosis of bacteria or particles of unorganized matter occurs not infrequently. It can sometimes be seen about bacterial foci and it is common in the neighborhood of old blood extravasations. Lambert and Hanes[2] have shown that carmine particles are ingested by some of the cells of *in vitro* cultures of sarcomatous tissue. But we need scarcely point out that in even the simplest of these instances the tissue is a congeries of cells of at least two sorts; namely, fibroblasts and endothelial cells from the blood and lymph channels. The latter are known to have phagocytic power. It is possible that their activity, with that of wandering cells, may account for the positive findings cited.

Method.

For our experiments bits of the heart and skeletal muscle from embryo chicks in the 3rd week of incubation, from embryo rats near to term, and from rats 2 to 5 days old were implanted in plasma of the appropriate species. Special care was taken to rule out the

[1] Rous, P., and Jones, F. S., *J. Exp. Med.*, 1916, xxiii, 549.

[2] Lambert, R. A., and Hanes, F. M., *J. Exp. Med.*, 1911, xiii, 495.

presence of blood, since some of the white cells might, by their phagocytic activity, have introduced confusion into the results. The tissue was washed free of blood by perfusing the animal with Locke's solution[3] injected into the heart, and the effectiveness of the washing, as well as the normality of the tissue, was controlled histologically. The plasma was centrifugalized at high speed, and the central portion drawn off for use through a fine pipette. The microscope showed it to be cell-free.

The cultures were made in small Petri dishes. Sometimes the undiluted plasma was used, and at other times it was mixed with from one to three parts of Locke's solution. After 24 to 48 hours' incubation of the cultures they were submitted to trypsin, and the freed cells were separated from the tissue fragments by filtration through gauze, were twice washed with Locke's solution by means of the centrifuge, and suspended in this fluid. 24 hour cultures yielded the best results. After longer periods the cells were apt to contain fat droplets.

In cultures made as described the muscle did not proliferate but there was an abundant outgrowth of connective tissue which, on digestion of the clot, furnished a thick suspension of living cells. The ability of these to take up particles of carmine was tested as follows: Finely ground carmine was added to the cell suspension, incubation carried on for 1 or for 2 hours, and the preparations were examined in the fresh, and in fixed films stained with methylene blue. Or the carmine was mixed directly with the plasma medium of the original culture, and when growth had taken place the cells were liberated with trypsin and examined for phagocytosis.

Experiments involving the phagocytosis of bacteria proved more troublesome, owing to difficulty in staining the preparations so that the presence of intracellular organisms and the character of the cells should both be brought out. A Gram-staining organism, *Staphylococcus pyogenes albus*, finally was selected, and the tissue cells were counter-stained with lithium carmine. This gave excellent results. Suspensions of freed cells were employed for the tests. With the cells of chicken connective tissue an antistaphylococcus serum was used,

[3] Locke's modification of Ringer's solution, but without sugar.

derived from chickens injected with cultures of the organism, and with rat cells, rat serum obtained in the same way. Leukocytes of the appropriate species were employed to determine the mixture of cell suspension, bacterial suspension, and serum, optimum for phagocytosis. In all the experiments control preparations without serum were made. Locke's solution was used throughout as a diluent. Incubation was for 2 hours, after which the cells were thrown down with the centrifuge, suspended in a little serum, and films taken.

The tests were many times repeated. The results were the same in all, irrespective of the species furnishing the cells, and whether they came from embryos or new-born animals. Both carmine particles and bacteria were phagocyted (Fig. 1), but always to a very small extent. Even in the best preparations it was necessary to go over many cells in order to find one phagocyte. The result cannot be attributed to unfavorable conditions in the mixtures, such for example as insufficient concentration of cells or bacteria, for the conditions were varied through a wide range, and in control preparations with leukocytes phagocytosis was profuse. Nor can it be laid to injury and death of the cells during the tryptic digestion and the subsequent incubation. Such treatment failed to lessen noticeably the phagocytic activity of leukocytes. The majority of the connective tissue cells were still alive at the conclusion of the tests, as shown by the refusal of the nuclei to stain with trypan blue.[4] Furthermore, as already stated, connective tissue cells freed with trypsin will proliferate when plated anew in plasma. The conclusion seems warranted that our technique was not at fault and that the phagocytic power of the cells derived from proliferating connective tissue is actually slight.

The phagocytosis of bacteria by the connective tissue cells occurred only in the presence of serum.

Nature of the Phagocytes.

The nature of the phagocytic cells remained to be decided. A study of stained films threw light on this question. In our previous paper[1] mention is made of the striking change in the form of cells which

[4] Rous and Jones, *J. Exp. Med.*, 1916, xxiii, 601.

occurs when they are freed from the clot in which they have been growing. The cells of connective tissue cultures, even those with an attenuated spindle shape, all take an approximately spherical form, and their processes merge in the general cytoplasm. But despite this approach to uniformity two sorts of cells, or rather two cell series, can be distinguished in stained preparations (Figs. 2 and 3). They have been figured in color in the paper referred to. With Wright's stain the cells of the smaller sort, which are much the more numerous, are characterized by a round or oval, pyknotic nucleus and a relatively scanty cytoplasm, while those of the larger kind have an oval, vesicular nucleus and abundant cytoplasm. The cytoplasm of both is somewhat basophilic, staining pale blue, and the smaller cells usually divide before it has reached any considerable bulk. Many of the latter closely resemble the lymphocytes of the blood, although in stained sections of the tissue from which they are derived lymphocytes are not evident. The observation may have a bearing on the long discussed problem of the origin of the cells resembling lymphocytes which appear in granulation tissue.

The differences between the two kinds of cells are striking and are still evident to some extent when a mixed suspension of them is plated out in plasma. The small kind then assume a slender, spindle form with long, and often attenuated processes, and the nuclei become oblong or indeed almost rod-shaped; whereas the large cells remain compact in form, as a rule irregularly stellate, with a few short processes, and the shape of the nucleus does not change. These differences are not always well maintained. The trypan blue test indicates that cells of the large kind die much more quickly when kept in Ringer's solution than do the small ones.

It is possible that here are young and old elements of a single sort; but the morphological evidence is against this and in favor of the assumption that the large cells are endothelial, and the more numerous, smaller ones are fibroblasts. Attempts to demonstrate fibroglia in the latter were unsuccessful, as was to have been expected because of their youth. The large cells, and these only, have in our experiments shown phagocytic activity,—a fact which is in keeping with the known character of endothelial cells.

SUMMARY.

By the tryptic digestion of cultures *in vitro* of avian and mammalian connective tissue, suspensions of individual, living cells have been obtained. Their ability to phagocyte carmine and bacteria has been tested. The great majority of them fail to take up either, but a few large cells are able to do so. They will ingest bacteria only when serum is present; that is, they require the interaction of opsonins. There is good reason to suppose that the phagocytic cells are endothelial in nature. Should they prove to be fibroblasts, like the other elements present, the fact will remain that the phagocytic power of fibroblasts is practically negligible. Their failure to ingest foreign matter *in vivo* is to be laid not to the obstacles offered by the solidity of the tissue they compose, but to an inherent lack of ability on their part. The phagocytosis of blood pigment, bacteria, etc., which takes place in granulation tissue *in vivo* is probably carried on wholly by endothelial cells and wandering cells.

EXPLANATION OF PLATES.

PLATE 22.

FIG. 1. Phagocyted staphylococci in cells derived from the connective tissue outgrowth from bits of the heart and skeletal muscle of young rats, cultivated *in vitro*. The cells belong to the larger of the two sorts figured in the illustrations which follow. Gram's stain followed by lithium carmine.

FIG. 2. The two sorts of cells liberated from connective tissue cultures by digestion with trypsin. Bits of the heart of a young rat were used for the cultures. Wright's stain.

PLATE 23.

FIG. 3. The same two sorts of cells but derived this time from cultures of the skeletal muscle of a young rat. In these cultures the intermuscular connective tissue alone had proliferated. Some of the large cells show fat droplets. Wright's stain.

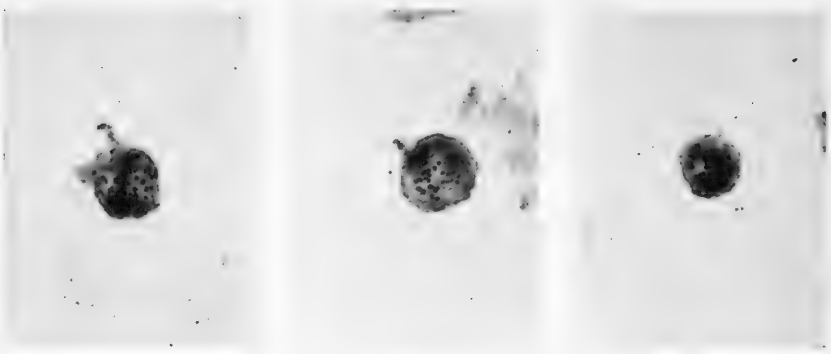

FIG. 1.

FIG. 2.

(Jones and Rous: Connective Tissue Cells.)

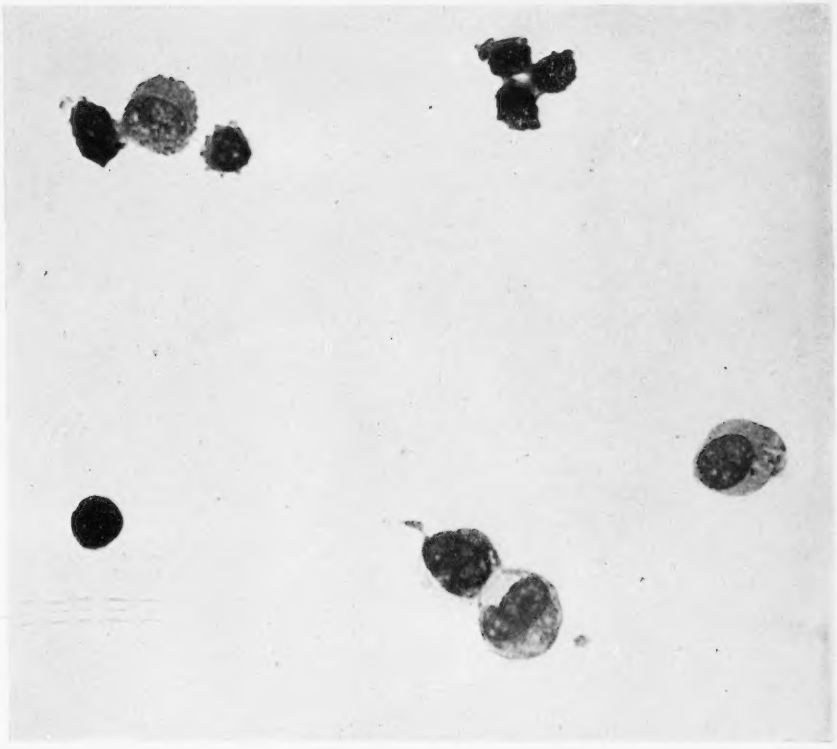

FIG. 3.

(Jones and Rous: Connective Tissue Cells.)